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May 10, 1948

SYMPOSIUM: AMINO ACID EXCRETION

RENAL CLEARANCES OF ESSENTIAL AMINO ACIDS

By LEMUEL D. WRIGHT*

Protein hydrolysates or amino acid mixtures are being employed widely today in clinical medicine. Present practices of administering these preparations either orally or parenterally in relatively large amounts have emphasized the need for additional information concerning the extent to which the various essential amino acids are lost in the urine.

There are a variety of approaches to the problems of the excretion of amino acids in the urine. We have selected to employ, in the studies to be reviewed in this paper, certain comparatively recently devised techniques of renal physiology whereby, under a variety of experimental conditions, the fate of the amino acids within the kidney may be fairly well traced. For a discussion of the underlying theory and technique involved in this field of research, reviews by Smith and others [1, 2] may be consulted.

In the experiments to be described, trained female dogs were employed. Infusion of the amino acids at definite rates was accomplished by the use of an electrically driven apparatus permitting constant rates of infusion. The amino acids were administered *via* the radial vein. Creatinine in amounts sufficient to permit the determination of reliable creatinine clearances was administered subcutaneously in aqueous solution. Blood samples were obtained from the femoral vein. All urine samples were obtained by catheterization. The bladder was washed out with a definite volume of water prior to and following the collection of urine samples in order to obtain quantitative urine collections.

Microbiological methods of assay have been employed in the determination of plasma levels and urine concentrations of the various amino acids studied. Determinations of the amino acids in plasma were carried out on protein-free filtrates prepared according to the method of Dunn *et al.*[3] Assays on suitably diluted urine samples were carried out without previous treatment. The method of Stokes *et al.*[4]

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Note. The Section of Geology and Mineralogy held a meeting on May 3, 1948, at which Professor Daniel T. O'Connell, Department of Geology, College of the City of New York, New York, N. Y., presented a paper entitled *Illustrations of Cloud Types*, illustrated by Kodachrome slides. No abstract of this has been received.

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TABLE 1

RENAL CLEARANCE STUDIES ON *l*(−)-TRYPTOPHANE

Dog 84, wt. 15.9 kg.

Time	Creatinine clearance	Urine flow	*l*(−)-Tryptophane				
			Plasma conc.	Amount filtered	Amount reabsorbed	Amount excreted	Clearance
Control: Post-absorptive state, but after priming dose of water							
hr. :min.	cc./min.	cc./min.	mg./cc.	mg./min.	mg./min.	mg./min.	cc./min.
0:10	56.7	2.40	0.014	0.76	0.76	0.002	0.18
0:20	63.9	2.95	0.014	0.83	0.83	0.002	0.19
Priming 0.75 mg./kg.—Maintenance 1.5 mg./kg./min.—Infusion 3 cc./min.							
0:45	52.8	2.80	0.051	2.69	2.68	0.009	0.19
0:55	49.0	2.40	0.063	3.09	3.07	0.015	0.24
Priming 0.75 mg./kg.—Maintenance 1.5 mg./kg./min.—Infusion 3 cc./min.							
1:30	44.3	2.25	0.091	4.03	4.00	0.033	0.36
1:41	46.3	2.08	0.118	5.46	5.42	0.045	0.39
Priming 1.0 mg./kg.—Maintenance 2.0 mg./kg./min.—Infusion 3 cc./min.							
2:12	46.0	1.82	0.164	7.54	7.47	0.072	0.44
2:23	48.8	1.64	0.158	7.71	7.64	0.075	0.47

employing *Streptococcus faecalis* R as the assay organism was used for the determination of arginine, histidine, methionine, leucine, isoleucine, and threonine. The method of Kuiken *et al.*[5] with *Lactobacillus arabinosus* was used for the determination of leucine, isoleucine, and valine. Lysine was measured with *Leuconostoc mesenteroides* P-60 according to Dunn *et al.*[6] Phenylalanine was determined with *Lactobacillus casei* by the procedure of Dunn *et al.*[7] Tryptophane was assayed with *Lactobacillus arabinosus* according to Greene and Black.[8]

The first phase of the work to be presented has been the determination of the renal clearances at normal and elevated plasma levels of each of the ten amino acids loosely referred to as "essential." In these initial experiments involving clearances of the individual amino acids, the plasma levels of each amino acid have been elevated to at least 10 times that of the fasting condition. It was reasoned that this level would be higher than any value possible to obtain following the administration of the amino acid in protein digests or complete amino acid mixtures. Subsequent investigations, to be discussed later, have demonstrated the correctness of this assumption.

Renal clearance data with tryptophane are shown in TABLE 1. The experiment described lasted about 2½ hours and involved the determination of tryptophane clearances in the post-absorptive animal, followed by similar clearances at various elevated plasma levels of tryptophane. Two calculations that are interrelated in their derivation, namely, the amount of tryptophane reabsorbed by the tubules in proportion to that filtered at the glomerulus and the clearance of tryptophane in comparison to that of creatinine, serve to demonstrate that tryptophane when administered alone is well reabsorbed by the renal tubules at all plasma levels feasible for study.

The amino acids leucine, isoleucine, valine, histidine, methionine, phenylalanine, and threonine have been examined in a similar fashion and resemble tryptophane in that tubular reabsorption is essentially complete following glomerular filtration from plasma levels up to at least 10 times that of the fasting state.[9-11]

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TABLE 2

RENAL CLEARANCE STUDIES ON *l*(+)-ARGININE

Dog 84, wt. 16.1 kg.

Time	Renal plasma flow PAH	Creatinine clearance	Urine flow	*l*(+)-Arginine Plasma conc.	Amount filtered	Amount reabsorbed	Amount excreted	Clearance
Control: Post-absorptive but after priming dose of water								
hr.: min.	cc./min.	cc./min.	cc./min.	mg./cc.	mg./min.	mg./min.	mg./min.	cc./min.
0:10	231.7	79.5	2.90	0.031	2.46	2.46	0.004	0.13
0:20	216.0	70.2	3.85	0.018	1.26	1.25	0.005	0.33
Priming 2.0 mg./kg.—Maintenance 2.0 mg./kg./min.—Infusion 3 cc./min.								
0:50	205.8	77.8	4.90	0.112	8.71	8.70	0.010	0.09
1:00		78.3	4.90	0.153	11.98	11.96	0.017	0.11
Priming 3.0 mg./kg.—Maintenance 6.0 mg./kg./min.—Infusion 3 cc./min.								
1:30		68.1	5.60	0.338	23.02	12.03*	10.99	28.3
1:40	177.5	57.8	5.80	0.458	26.47	12.80*	13.67	29.8
Priming 4.0 mg./kg.—Maintenance 10 mg./kg./min.—Infusion 3 cc./min.								
2:10	174.6	60.7	6.64	0.680	41.28	10.75*	30.53	44.9
2:20		63.6	6.78	0.720	45.79	8.42*	37.37	51.9

* Tm = 12.02, 12.80, 10.75 and 8.42 (average 11.00) mg./min.

TABLE 2 summarizes renal clearance studies with arginine. As the plasma level of this amino acid was elevated, resulting in commensurate increases in the amount of amino acid filtered, there was encountered a plateau in the amount of the compound that may be reabsorbed amounting to about 11 mg./min. This value is spoken of as maximal rate of tubular reabsorption or Tm.[10]

The amino acid lysine is quite similar to arginine in being poorly reabsorbed following filtration from elevated plasma levels. The Tm for lysine is approximately 13 mg./min. for a dog of about 15 kilos.[10]

This phase of the work may be summarized by saying that eight of the essential amino acids leucine, isoleucine, valine, methionine, histidine, threonine, phenylalanine, and tryptophane are reabsorbed well at elevated plasma levels while two, arginine and lysine, are poorly reabsorbed at elevated plasma levels.

Having demonstrated that the individual essential amino acids vary with respect to the extent to which they are reabsorbed by the kidney following filtration from elevated plasma levels, it has been of interest to inquire what effect, if any, the reabsorption of one essential amino acid may have on the reabsorption of another related or unrelated amino acid. The next phase of this discussion describes certain studies wherein various *pairs* of amino acids were administered together and their competition for reabsorption by the renal tubules investigated.[12] These studies have involved the coadministration of (a) two amino acids *well* reabsorbed at high plasma concentration, (b) two amino acids relatively *poorly* reabsorbed at high plasma levels, or (c) two amino acids one of which was *poorly* reabsorbed and the other *well* reabsorbed when each was administered alone.

TABLE 3 illustrates an instance of competition for reabsorption between the amino acids arginine and lysine. In the first phase of the experiment, the effect of arginine, at filtration loads sufficient to measure its Tm, on the reabsorption and clearance of endogenous lysine was studied. In the second phase of the experiment, the mutual effect of the two com-

TABLE 3

COMPETITION BETWEEN ARGININE AND LYSINE FOR TUBULAR REABSORPTION

Dog 365, wt. 13.9 kg.

Creatinine clearance	Urine flow	*l*(+)-Arginine				*l*(+)-Lysine			
		Plasma conc.	Amount filtered	Amount reabsorbed	Clearance	Plasma conc.	Amount filtered	Amount reabsorbed	Clearance
Arginine dosage: Priming, 4.0 mg./kg., i.v.; maintenance, 7.0 mg./kg./min., i.v. for the duration of the experiment. Infusion rate, 3.0 cc./min. Equilibration period of arginine infusion before initial clearance, 25 min.									
cc./min.	cc./min.	mg./cc.	mg./min.	mg./min.	cc./min.	mg./cc.	mg./min.	mg./min.	cc./min.
67.7	7.7	0.31	20.85	9.67	36.29	0.048	3.25	1.75	31.3
67.0	8.2	0.38	25.73	9.46	42.37	0.051	3.42	1.93	29.2
Lysine dosage: Priming, 4.0 mg./kg., i.v.; maintenance, 7.0 mg./kg./min., i.v. Arginine dosage: Maintenance, 7.0 mg./kg./min., i.v. Equilibration period of arginine plus lysine infusion before next clearance, 25 min.									
58.0	10.4	0.54	31.15	3.05	52.82	0.40	23.03	3.13	50.10
59.7	10.3	0.61	36.60	5.10	51.38	0.46	27.70	2.64	54.00

pounds at elevated plasma concentration on their respective reabsorption capacities was studied.

The results of TABLE 3 show that the reabsorption of arginine at elevated plasma concentration, or filtration load, suppressed the tubular reabsorption of endogenous lysine significantly. This effect is the more apparent if it is recalled that, at an endogenous plasma concentration of lysine alone, its clearance was 0.14 cc./min. as compared with clearances of about 30 cc./min. in the present study at similar plasma concentrations.

At elevated plasma concentrations of arginine and lysine simultaneously administered, Tm values of 3-5 mg./min. for arginine and 2-3 mg./min. of lysine were encountered. If it is recalled that the Tm for lysine administered alone is about 13 mg./min., the conclusion seems justified that the reabsorption of one amino acid of this pair results in impaired reabsorption of the other member. In passing, it might be mentioned that it has been possible, in at least one experiment, to inhibit lysine reabsorption completely by the administration of arginine. In other words, all the lysine filtered by the glomerulus was eliminated in the urine.

Other instances of competition of amino acids for reabsorption or interference of one amino acid with tubular reabsorption of another amino acid have been noted. This phenomenon has not been observed, however, with all the amino acid pairs investigated. Apparently, there exist certain pairs of amino acids where competition for reabsorption is demonstrable and certain pairs of amino acids for which no competition can be shown. We have attempted to correlate this information in terms of what it may mean with respect to mechanisms by which amino acids in general are reabsorbed by the kidney tubules. We have postulated that those amino acids which can compete with each other for reabsorption are reabsorbed by a common mechanism. Furthermore, where competition for reabsorption cannot be demonstrated for two amino acids, it would follow that these two amino acids belong to separate groups with respect to mechanisms of tubular reabsorption. FIGURE 1 summarizes the results of such an interpretation. One reabsorption mechanism appears to be concerned with reabsorption of the basic amino acids arginine, histidine, and lysine. A second reabsorption mechanism may be postulated to exist for at least the monoamino-monocarboxylic amino acids leucine and isoleucine. Apparently, at least a third

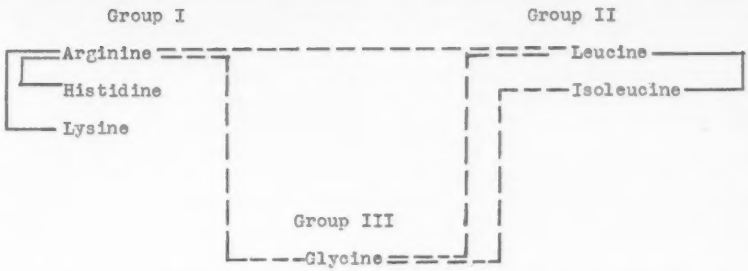

FIGURE 1. Classification of amino acids into groups with respect to probable mechanism of reabsorption. Solid lines indicate established interference; broken lines indicate no demonstrable interference.

mechanism of reabsorption exists, for it has been impossible to demonstrate a relationship between glycine and members of either group I or II.

It is obvious from our knowledge of the biological value of proteins that the administration of single amino acids or incomplete amino acid mixtures, except in certain isolated instances, is of no practical value. A few experiments have been carried out on the renal clearances of certain amino acids following the intravenous administration of the ten essential amino acids or an acid hydrolysate of casein reinforced with tryptophane. It should be appreciated that the amount of amino acid mixture or protein hydrolysate that can be administered intravenously to an animal is quite low. Such preparations, when administered at too great a rate, usually cause severe nausea and oliguria. Accordingly, although we can administer an amino acid mixture or protein hydrolysate *in amounts as large as we have been able to do with single amino acids* because any *one* amino acid comprises only a small fraction of a complete protein, we have not been able in this way to elevate the plasma levels of various amino acids to those levels readily obtained by single amino acid administration.

TABLE 4

RENAL CLEARANCE STUDIES OF ARGININE AND THREONINE ADMINISTERED IN A MIXTURE OF THE TEN ESSENTIAL AMINO ACIDS

Dog 370, wt. 14.6 kg.

Creatinine clearance	Urine flow	*l*(+)-Arginine				*dl*-Threonine			
		Plasma conc.	Amount filtered	Amount reabsorbed	Clearance	Plasma conc.	Amount filtered	Amount reabsorbed	Clearance
Control: Post-absorptive but after priming dose of water									
cc./min.	cc./min.	mg./cc.	mg./min.	mg./min.	cc./min.	mg./cc.	mg./min.	mg./min.	cc./min.
75.5	6.6	0.020	1.55	1.54	0.40	0.038	2.94	2.92	0.43
69.2	4.0	0.017	1.18	1.17	0.65	0.032	2.21	2.19	0.52
Amino acid mixture dosage: Priming 4.0 mg./kg., i.v.; Maintenance 4.0 mg/kg./min. Infusion rate 6 cc./min. Equilibration period of infusion before initial clearance 30 min.									
76.1	3.8	0.026	1.98	1.96	0.61	0.059	4.49	4.45	0.71
78.9	3.4	0.032	2.52	2.50	0.61	0.069	5.44	5.39	0.78
Amino acid mixture dosage: Priming 8.0 mg./kg., i.v. Maintenance 8.0 mg./kg./min. Infusion rate 6 cc./min. Equilibration period of infusion before initial clearance 30 min.									
77.2	1.2	0.039	3.01	2.98	0.75	0.095	7.33	7.14	1.95
62.8	0.5	0.040	2.51	2.48	0.83	0.102	6.41	6.23	1.72

TABLE 4 summarizes an experiment wherein a mixture of the ten essential amino acids was administered in the indicated amounts. Arginine was selected for study because, from our studies of the renal clearances of the separate amino acids, arginine was found to have the lowest Tm and, consequently, might be expected to be the first amino acid to appear in the urine. Threonine was studied because it has been observed to be one of the amino acids excreted in greatest amount following filtration from a fasting plasma level. When the ten essential amino acids were administered at approximately the maximal tolerated amount, there was no indication that the maximal rate of tubular reabsorption of either arginine or threonine had been even approached.

TABLE 5 summarizes an experiment

TABLE 5

RENAL CLEARANCE STUDIES OF ARGININE AND THREONINE ADMINISTERED IN THE FORM OF ACID HYDROLYZED CASEIN PLUS TRYPTOPHANE

Dog 84, wt. 17.8 kg.

Creatinine clearance	Urine flow	*l*(+)-Arginine				*dl*-Threonine			
		Plasma conc.	Amount filtered	Amount reabsorbed	Clearance	Plasma conc.	Amount filtered	Amount reabsorbed	Clearance
Control: Post-absorptive but after priming dose of water									
cc./min.	cc./min	mg./cc.	mg./min.	mg./min.	cc./min.	mg./cc.	mg./min.	mg./min.	cc./min.
101.3	6.95	0.010	1.01	1.00	1.27	0.063	6.38	6.37	0.20
97.2	6.95	0.012	1.17	1.16	0.78	0.063	6.12	6.11	0.12
Casein dosage: Priming 4.0 mg./kg., i.v.; Maintenance 4.0 mg./kg./min. Infusion rate 3 cc./min. Equilibration period of infusion before initial clearance 30 min.									
90.5	6.80	0.013	1.17	1.16	1.02	0.066	5.97	5.95	0.34
90.4	7.10	0.017	1.54	1.52	1.27	0.072	6.51	6.47	0.48
Casein dosage: Priming 8.0 mg./kg., i.v.; Maintenance 8.0 mg./kg./min. Infusion rate 3 cc./min. Equilibration period of infusion before initial clearance 30 min.									
92.4	8.55	0.017	1.57	1.54	1.78	0.067	6.19	6.14	0.75
89.4	8.20	0.019	1.70	1.67	1.68	0.072	6.44	6.38	0.75

where an acid hydrolysate of casein plus tryptophane was administered to a dog at the maximal tolerated dose. Here, as with the amino acid mixture, arginine and threonine were not eliminated in excessive amounts in the urine.

In summarizing this paper, the following conclusions may be made:

(1) Amino acids vary with respect to the efficiency with which they are reabsorbed following filtration from elevated plasma levels.

(2) Certain amino acids may compete with each other for reabsorption by the renal tubules. Such competition studies have indicated the existence of more than one functional mechanism involved in the reabsorptive process of amino acids.

(3) The intravenous administration of amino acid mixtures or protein hydrolysates to the intact animal does not result in plasma levels of the individual amino acids such that their maximal rate of tubular reabsorption is exceeded.

(4) Plasma levels of the various amino acids following the intravenous administration of amino acid mixtures or protein hydrolysates are too low for marked competitive inhibition of reabsorption to be encountered.

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THE UTILIZATION OF AMINO ACID MIXTURES BY DOGS

By R. H. SILBER, E. E. HOWE, AND C. C. PORTER*

Mixtures of 9 or 10 "essential" amino acids have been found to promote growth in weanling rats.[1] Recently, it has been demonstrated [2] that the same amino acids also satisfy the repletion requirements of protein-depleted adult rats. Rose and Rice [3] reported that the amino acid requirements of the adult dog are qualitatively similar to those of the weanling rat. The ten "essential" amino acids of Rose have also been found to satisfy the requirements of the adult dog for plasma protein production, nitrogen balance, and weight maintenance.[4]

When paired-feeding is not employed, the growth of rats supplied amino acids does not equal the growth of rats fed high-quality protein.[5] The superiority of protein is generally assumed to depend upon the presence of dietary factors other than amino acids in the protein.

The Growth and Maintenance of Dogs Fed Mixtures of Amino Acids, Free of the D-Forms and Peptides. A litter of six 12 week-old Beagle puppies, weighing about 3.5 kg. each, was divided into 3 groups—2 pups were fed a protein-free diet,[6] 2 received the same diet with 20 per cent amino acids added, and 2 received 20 per cent Labco casein in the diet. Paired feeding was employed.

For a period of 12 weeks, the amino acid-fed pups gained an average of 0.4 kg. per week, whereas the pair-fed casein control pups gained 0.43 kg. per week. The pups that received protein-free diet lost about 0.5 kg. each in 4 weeks and were then offered the amino acid diet. A gain in weight of 0.26 kg. per week resulted during the 12-week repletion period.

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Blood chemistry, urinary analyses, and bromsulfalein retention tests revealed only one significant difference between the pups fed casein or amino acids. The feeding of amino acids favored the production of serum albumin and liver protein, relative to casein feeding.

In the first 3 or 4 days of the experiment, the pups consumed their amino acid diets rapidly and vomited shortly thereafter. Subsequently, they learned to consume the diet in small portions and succeeded in retaining sufficient amounts for growth. It is important to note that, if the pups had not learned to consume the diet in this way, it might have been considered inadequate in the usual nutritional sense, although all essential factors were present.

Since both dogs and rats have shown evidence of a physiological disturbance (nausea or diarrhea) when amino acid mixtures or protein hydrolysates are given orally, it seems logical to assume that there may be a physiological limitation of the voluntary intake of amino acid-containing diets, and may therefore explain, at least in part, the inability of animals to grow as rapidly on amino acid diets as on protein diets supplied *ad libitum*.

The maintenance of adult dogs fed or injected with amino acids as the sole source of dietary nitrogen was also studied. Two adult dogs were fed 1.5 gm. of casein (200 mg. N) and 80 calories per kg. per day for a period of 64 days. Two other dogs received an equivalent amount of nitrogen in the form of an amino acid mixture, in the diet for 40 days and by vein for the subsequent 24 days. Two additional dogs served as negative controls and received a protein-free diet throughout the test period.

Body weights and nitrogen balance were maintained in the dogs fed either amino acids or casein. Blood chemistry and urinary analyses revealed only minor differences between the animals. However, as in the pup experiment, amino acid feeding favored serum albumin and liver protein formation, relative to casein feeding.

The Excretion of Amino Acids and Peptides by Dogs Fed Protein Hydrolysates and Amino Acid Mixtures Orally or Intravenously. Six preparations, designed for intravenous alimentation of man, were given orally or parenterally to dogs previously maintained at a low level of protein nutrition by the feeding of 120 mg. of lactalbumin nitrogen per kg. per day. Each preparation was administered to each of 3 or 4 dogs for a period of 4 days and urines were collected quantitatively for analysis. The excretion of amino acids, peptides, and total nitrogen was compared with the excretion found after the oral administration of an equivalent amount of lactalbumin nitrogen.

Four of the preparations contained 25 to 45 per cent of their amino acids in peptide form. Two contained no peptides. After oral administration, 0.3 to 4.3 per cent of the free amino acids and 2 to 10.5 per cent of the peptides were lost in the urine. Nitrogen balance experiments* in dogs showed that all preparations were approximately equal to casein in biological value, with 61 to 73 per cent of the nitrogen retained in the body after oral administration.

When the same preparations were infused intravenously at a rate of 2 mg. N per kg. per min., 5 to 20 per cent of the free amino acids and 18 to 45 per cent of the peptides administered were found in the urine. The dogs infused with the preparation which had the highest peptide content also excreted the largest fraction of peptides (45 per cent). Furthermore, all of the nitrogen of this preparation was lost in the urine, although it had been previously demonstrated that, after oral administration, its biological value (nitrogen retention) was equal to that of the other five preparations. Such a preparation would not necessarily be valueless for intravenous therapy in man, but the results emphasize the necessity of proving that a

* The design of the nitrogen balance experiments was the result of a group effort (U.S.P. Subcommittee, headed by Drs. J. B. Allison and D. V. Frost) and will be reported as such at a later date.

preparation designed for intravenous use is available by this route when given to the species for which it is intended.

When the daily intake of protein hydrolysate or amino acid mixture was infused very rapidly (15 min.), only 25 per cent of the nitrogen was retained. This was due to an increased excretion of urea and ammonia nitrogen, not to excessive loss of amino acids in the urine, as suggested by Elman.[7] A four-fold increase in the rate of infusion (2 mg. N per kg. per min. to 8 mg. N per kg. per min.) caused a moderate increase in the loss of free amino acids in the urine (5 to 15 per cent), but had no effect on the excretion of peptides (40 per cent). This suggests that the loss of peptides in the urine is not primarily a problem of kidney threshold—that certain peptides cannot be utilized after intravenous administration, whether given at slow or fast rates. In this connection, it is of interest that some of the peptides were not utilized even after oral feeding. These data support the finding of Christensen[8] that intravenously administered amino acids are more readily utilized than peptides.

The Relationship Between Intake and Excretion of Essential Amino Acids in Dogs. In this study, 3 different amino acid mixtures were infused into 2 normal dogs at rate of 6 mg. N per kg. per min. and the urines were assayed by the methods of Stokes[9] for the essential amino acids. The total nitrogen intake was 200 mg. N per kg., and this was the only source of dietary nitrogen supplied during the one-day experiment. Approximately 10 per cent of the amino acids administered were excreted in the urine.

After the first experiment, the amino acid mixture was altered on the hypothesis that a disproportionately large excretion of any one essential amino acid indicates that it is present in excess in the mixture, and, conversely, that a disproportionately small excretion indicates that it is present in an inadequate amount. The concept is essentially the same as that involved in vitamin saturation tests, but is applied here to the relative needs of the body for the various amino acids.

As shown in TABLE 1, it has been possible to greatly decrease or eliminate the differences between the proportions administered and those excreted. Thus, application of the above hypothesis apparently makes it possible to determine the composition of an amino acid mixture which, after infusion into normal dogs at a uniform rate, will be excreted in essentially the same proportions (or pattern) as in the mixture given.

If the capacity of the absorptive mechanism(s) of the renal tubules is exceeded in these experiments, one amino acid (or more) may tend to "spill" into the urine. If this occurs, it might not be possible to determine the proportion of that amino

TABLE 1

THE PROPORTIONS OF ESSENTIAL AMINO ACIDS IN THE MIXTURES AND IN THE URINES OF DOGS AFTER INFUSION

Amino acid	Experiment 1		Experiment 2		Experiment 3	
	Mixture, %	Urine, %	Mixture, %	Urine, %	Mixture, %	Urine, %
Arginine	9.2	10.0	7.1	3.5	8.1	3.5
Histidine	4.2	9.9	2.1	5.7	1.0	1.4
Isoleucine	10.8	4.1	13.6	9.0	14.4	10.2
Leucine	24.3	12.4	31.4	25.6	33.2	34.2
Lysine	13.6	33.4	6.9	12.9	3.3	5.3
Methionine	8.5	3.0	9.9	6.9	12.0	11.6
Phenylalanine	12.1	4.8	11.9	10.5	13.1	8.8
Threonine	7.5	19.0	4.1	13.3	2.8	9.3
Tryptophane	1.3	0.3	1.8	1.2	2.0	2.1
Valine	8.5	4.2	11.0	11.6	10.0	13.3

acid which will be repeated in the urine, due to the low threshold. Threonine may be such an exception, since extrapolation of the values obtained with this amino acid indicate that threonine will be excreted in a larger proportion in the urine regardless of the proportion in the infused mixture. Another interpretation of this result is that threonine is not needed by the dog under these conditions, which seems unlikely.

The specificity of the assay procedures for amino acids in urine is another limiting factor. It may be necessary to revise these results as more knowledge of the assay methods is obtained. The excretion of ketoacids of the essential amino acids would not complicate the experiment if the microbial assays detect these forms.

Since low renal thresholds for certain amino acids could seriously limit their utilization by the tissues, experiments are now being designed to minimize the role of the kidney, that is, a lower intake and a lower rate of infusion are being employed. Furthermore, the dogs are maintained for two weeks at a basal level of protein nutrition prior to infusion for a period of 4 days.

It remains to be determined how accurately this approach reflects the relative amino acid needs of the animal. If the small fraction of the infused mixture which is excreted in the urine (about 10 per cent) has the same relative composition, or pattern, of amino acids as the infused mixture, it follows that the remaining 90 per cent, having the same relative composition, was metabolized in the body. This must reflect, at least in part, the needs of the animal under the conditions of the experiment.

Whether this approach reveals specific amino acid needs or merely an increased need for certain amino acids, it may be useful in the future in designing amino acid mixtures for specific types of patients. Thus, a study of this nature, if carried out in a group of similar patients, may lead to the "tailoring" of an amino acid mixture for such patients.

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URINARY EXCRETION OF INDIVIDUAL AMINO ACIDS ON NORMAL AND LOW PROTEIN DIETS

By STANLEY W. HIER*

The precise measurement of the quantities of amino acids excreted in the urine is complicated by a number of factors. First, the amino acids are present in conjugated and peptide forms as well as in free form. Second, numerous derivatives and metabolites of the amino acids are also excreted. Third, the presence of ammonia, urea, and salt may influence chemical or microbiological tests.

Our experience in this field has been limited to microbiological methods which we have applied successfully to blood plasma, spinal fluid, and lymph. The presence of urea in urine has created a

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special problem in the handling of this body fluid, particularly for the determination of the free amino acids present in normal urine. Dilutions of only 1-2 or 1-4 are permissible in some cases to measure the small amounts of free amino acids present. At this low dilution, the amount of urea will be between 5-12 mg./cc. of the assay sample. This concentration has a marked depressing effect upon the growth of test organism as measured by acid production. Part of this effect is due to the formation of ammonia upon autoclaving the media, since the pH is markedly increased as the concentration of urea is increased. However, urea as such, introduced aseptically, will also inhibit growth.

The values we report for free amino acids were obtained by using only those levels of sample which supplied less than 3 mg. of urea per tube. Nevertheless, one hesitates to state that these values are absolute, in view of the uncertainty as to the influence of the urea. We feel that the determination of excretion of the total amount of the individual amino acids excreted has a much higher degree of significance because the urea effect is eliminated. The urine is made 2 N with HCl and autoclaved at 15 at. pressure for 5 hours. For tryptophane, alkaline hydrolysis is employed. In this way, all the urea is eliminated and very good recoveries are obtained. The procedure is not particularly satisfactory for cystine, which is destroyed by acid hydrolysis. The tryptophane analysis also creates a problem, since the extent of racemization is unknown.

With these considerations in mind, I should like to present data which have been obtained in studies on the influence of diet on amino acid excretion.

On normal diets[1] (TABLE 1), it may be seen that the mean value for excretion of aspartic acid in free form is 1.3 mg./day, while histidine is excreted at the rate of 188 mg./day. The amount excreted of the remaining amino acids shown falls in between. When the totals (free and combined) are considered, the range is from 20.3 mg./day for isoleucine to 351.4 mg./day for glutamic acid.

The range for individuals may be seen to be at least 3-fold or greater for each amino acid. Only a small portion of the glutamic acid and aspartic acid is excreted in free form, while practically all the histidine is excreted in free form (or at least microbiologically active form). The other amino acids fall intermediate between these in the relative amounts of

TABLE 1

URINARY EXCRETION OF "FREE" AND "COMBINED" AMINO ACIDS BY EIGHTEEN SUBJECTS ON NORMAL DIETS

(Reported as mg. per 24 hours)

	Free		Total		Combined		Per cent combined form of total	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Arginine	11.0–36.2	21.3±6.9	12.2–45.0	23.7±7.9	0–13.8	6.6	0–52.9	24.2
Aspartic acid	0–23.6	1.3±0.6	87.4–258.8	164.5±46.7	82.7–258.8	163.2	82.1–100	99.2
Cystine	45.2–138.0	87.7±25						
Glutamic acid	0–63.7	35.8±19.2	102.4–769.5	351.4±151.4	72.6–708.7	315.6	79.0–100	89.8
Histidine	60.3–378.0	188.3±99.2	65.4–438.8	203.3±101.1	5.1–123.2	40.5	4.8–34.5	14.9
Isoleucine	0–20.1	5.9±4.5	11.8–33.4	20.3±5.5	3.9–30.3	14.4	18.3–100	71.0
Leucine	3.8–18.5	9.6±3.3	11.9–40.0	21.2±6.6	3.5–29.6	12.3	29.4–81.8	55.6
Lysine	18.2–88.2	33.6±16.9	35.6–166.0	73.2±29.4	15.4–77.8	43.0	23.4–74.8	57.1
Methionine	4.1–13.5	7.8±2.9	4.1–15.0	8.6±2.8	0.5–6.3	2.3	4.7–60.5	24.6
Phenylalanine	7.5–34.0	16.4±7.1	10.3–45.4	23.3±7.9	3.1–16.9	8.4	14.4–66.6	35.2
Proline	3.7–14.8	8.5±2.9	23.1–62.1	42.8±12.9	18.1–54.4	34.3	65.1–89.0	80.2
Threonine	12.4–49.5	24.4±10.9	14.8–84.4	53.8±19.5	18.8–53.8	31.3	41.7–67.9	58.0
Tryptophane	8.5–56.0	24.6±11.3	11.2–86.1	41.4±17.5	0.6–28.3	18.4	1.3–71.8	41.9
Tyrosine	10.5–43.9	20.8±1.7	23.4–100.3	52.5±18	5.9–60.3	31.7	25.2–75.1	60.4
Valine	0–7.5	4.5±2.2	11.0–30.0	19.8±5.6	6.1–28.2	15.3	55.4–100	77.3

free and combined amino acids excreted.

When the values for total amino acid excretion for individual amino acids obtained by us are compared to those of Dunn,[2] excellent agreement is obtained. The values reported by Steele[3] for free amino acids excreted on various diets do not agree as well. Part of this, we believe, is due to differences in diet, but principally the dilutions used by Steele are quite different from ours and, therefore, the influence of urea and other urinary constituents may vary.

It is evident, from our data as well as from those of Steele, that methionine is apparently excreted to a much smaller extent than cystine. Even though the egg protein diet supplies considerably more methionine than the soy bean diet, the excretion is the same on both diets for cystine and methionine. Apparently methionine is utilized or deaminated more readily than cystine.

After determining the normal excretion of amino acids, we were interested in determining the influence of low protein diets on excretion of total amino acids. TABLE 2 shows 3 diets fed to healthy normal young women for 11 days on Level B and D and 6 days on Level E. The protein in both B and D is a balanced mixture of meat, potatoes, oats and bread but given in limited quantity. Level E contained no protein except in the cookies. The results are shown in TABLE 3. In all cases except phenylalanine and tyrosine, there is a decrease in the excreted amino acids on the low protein diets as compared to normal diets. There is, however, no direct correlation between intake and amino acid excretion since the excretion on 0.2 gm. N/day is not different from that on 2.5 gm. N/day. This may be due to the limited duration of the experiment and need for longer depletion periods.

Sauberlich and Baumann,[4] in rat experiments, have shown that, in the rat, the amino acids excreted daily are proportional to the protein intake. They fortified the casein with cystine so that the quality of the protein was not involved, but only quantity. They also showed that the percentage of ingested amino acids excreted on the low protein intake is slightly

TABLE 2

FOOD INTAKE, PROTEIN CONTENT, AND CALORIC VALUE OF THREE DIETS (2.5, 1.3 AND 0.2 GM. NITROGEN) FED IN PROTEIN STUDY

Foods	Level B (2.5 gm N_2)			Level D (1.3 gm N_2)			Level E (low N_2)		
	Amt. of food gm.	Protein gm.	Calories*	Amt. of food gm.	Protein gm.	Calories	Amt. of food gm.	Nitrogen gm.	Calories
Apple sauce	100	0.2	80.0	100	0.2	80.0			
Lemon juice	100 ml.	.....	33.0	100 ml.	.....	33.0	100 ml.	.....	33.0
Lettuce	30	0.4	5.4	30	0.4	5.4			
French dressing (vinegar and spice)	10 ml.	.....	.....	10 ml.	.....	.....			
Salad oil	10 ml.	.....	90.0	10 ml.	.....	90.0			
Beef-chuck	11	2.0	24.0	8.3	1.5	18.1			
Butter fat	4.4	.....	39.6	3.4	.....	30.6			
Cream (coffee)	64.2	1.9	133.5	48.6	1.4	101.1			
Potatoes	13.3	0.3	12.5	10.1	0.2	9.5			
White bread	91.0	8.4	237.5	68.9	6.3	179.8			
Oats, rolled	10.4	1.5	41.2	7.8	1.1	30.9			
Fondant	65.0	.....	231.0	65.0	.....	231.0	65.0	.....	231.0
Stuart formula tablets, 2									
Cookies	276.7	.....	1141.0	276.7	.....	1141.0	276.7	.....	1141.0
Total		14.7	2068.7		11.1	1950.4			1405.0

* Calculated values from: Bradley, Table of Food Values.

TABLE 3

EFFECT OF LOW PROTEIN DIET ON URINARY EXCRETION OF AMINO ACIDS

(Determined after hydrolysis)

Amino acid	Normal diet	2.5 gm. N/day (11 days)	1.3 gm. N/day (11 days)	0.2 gm. N/day (6 days)
Arginine	23.7	17.3	19.9	14.9
Aspartic acid	164.5	100	82.1	81.8
Glutamic acid	351.4	194	169	168
Histidine	203.3	106	75.5	97.0
Isoleucine	20.3	9.2	11.0	8.6
Leucine	21.2	14.9	15.6	13.6
Lysine	73.2	36.3	36.7	29.3
Methionine	8.6	4.5	3.9	3.7
Phenylalanine	23.3	26.6	29.3	20.7
Threonine	53.8	39.2	29.5	32.4
Tryptophane	41.4	9.3	6.3	6.4
Tyrosine	52.5	48.9	43.3	41.9
Valine	19.8	14.1	12.3	10.9

higher than on the high protein intake. The range is from about 0.3-1.58 per cent. In the mouse, however, the excretion is much higher, ranging from 3-9 per cent of the ingested protein. This is partly explained by the fact that more of the amino acids are excreted in peptide form by the rat than by the mouse. There is a 4-fold increase in amino N after hydrolysis in the rat, but only 2-fold in the mouse.

The work of Pearce[5] demonstrates the additional fact that, on a protein diet deficient in one amino acid, the excretion of all amino acids is higher than that on a normal good protein diet. This is due to the inability of the organism to utilize the inadequate mixture of amino acids.

Summary

Sixteen amino acids assayed microbiologically were found to be present in some or all of normal human urines studied in free form. All were found to be present after hydrolysis to release peptides or conjugates.

Aspartic acid is almost completely bound, while 90 per cent of the glutamic acid is bound or unavailable to the organism until urine is hydrolyzed. Histidine, arginine and probably cystine, on the other hand, are present in urine principally in the free form (75-85 per cent). The remaining amino acids are present in approximately equal amounts of the free and combined forms.

Total excretion of aspartic acid, glutamic acid, and histidine is highest (160-350 mg./day), while excretion of methionine is lowest (8.6 mg./day). The excretion of the other amino acids falls in between.

Human subjects excrete smaller amounts of the amino acids studied as protein intake is decreased. Phenylalanine and tyrosine appear to be exceptions to this rule. Similar results were obtained with rats and mice. However, if an inadequate protein is fed to rats, the excretion of other individual amino acids is increased. The excretion of amino acids by human subjects is quite low on normal diets, since, with the exception of histidine, less than 3 per cent of the ingested amino acids are excreted.

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THE URINARY EXCRETION OF AMINO ACIDS IN MAN

By RICHARD D. ECKHARDT, ARNOLD M. COOPER, WILLIAM W. FALOON, AND CHARLES S. DAVIDSON*

There is no adequate substitute for a well-balanced oral diet. However, occasions arise when a nutritious diet cannot be adequately eaten, digested or absorbed and parenteral protein hydrolysate therapy is indicated. Numerous reports have been published concerning the nutritional adequacy of protein hydrolysates, their clinical tolerance, and their freedom from antigenicity. However, few studies have dealt with the excretion of amino acids in the urine following infusions of protein hydrolysate in man.

The experiments reported here were undertaken to determine the extent of the renal loss of amino acids after their intravenous infusion in man. During the course of these investigations, the excretion of amino acids by normal subjects on *ad libitum* diets and on diets of varied protein content was determined and will be summarized. Further, observations on the excretion of amino acids following infusions of amino acids in patients with active cirrhosis of the liver and Wilson's disease will be compared with results obtained in normal subjects.

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Excretion of Amino Acids by Normal Subjects Eating ad libitum. The 24-hour urines of 8 normal adult subjects eating *ad libitum* of diets estimated to contain an average of 105 grams of protein daily were assayed (microbiological method of Stokes *et al.*[1]) for the 8 amino acids "essential" for man[2] and for arginine and histidine. The results tabulated in TABLE 1 are in general agreement with other published values employing microbiological methods.[3–5]

The urinary excretion of alpha amino nitrogen (gasometric ninhydrin method of Van Slyke, MacFadyen, and Hamilton[6]) by the 8 normal subjects averaged 158 mg. for the 24-hour period, a higher value per hour (6.6 mg.) than that of fasting specimens, which averaged 4.3 mg. per hour. Thus, the food ingested by the subjects increased the fasting excretion of alpha amino nitrogen from 103 mg. (4.3 mg. x 24 hours) to 158 mg. daily. The loss of 55 mg. of alpha amino nitrogen after consuming 105 grams of protein represents excretion into the urine of only 0.4 per cent of the amino nitrogen ingested.* This

* Assuming the food protein to contain 16 per cent total nitrogen, and the total nitrogen to be 80 per cent alpha amino nitrogen.

TABLE 1

URINARY EXCRETION OF 10 "ESSENTIAL" AMINO ACIDS AND OF "FREE" ALPHA AMINO NITROGEN IN THE NORMAL, IN LIVER DISEASE, AND IN WILSON'S DISEASE (*Diet ad libitum*)

	Normals (Av. 8 subjects) mg./24 hours	Liver Disease (Av. 7 patients) mg./24 hours	Wilson's Disease (Av. 5 patients) mg./24 hours
Arginine	9.5	10.6	46.6
Histidine	163	122	393
Isoleucine	13.8	4.5	24.4
Leucine	7.2	11.4	72.6
Lysine	46.8	28.0	218
Methionine	4.4	10.5	19.4
Phenylalanine	14.0	20.4	119
Threonine	20.9	26.4	261
Tryptophane	12.7	21.9	59.2
Valine	7.4	6.8	62.3
"Free" Alpha Amino Nitrogen	158 (120 to 199)	175 (84 to 258)	399 (204 to 640)

value is identical with that observed to follow the oral ingestion of 50 grams of whole casein.[7]

Excretion of Amino Acids by a Normal Subject Receiving Diets of Varied Protein Content. Since food by mouth produced only a moderate increase in the excretion of amino nitrogen above that excreted in the fasting state, the effect on the excretion of amino acids by varying the protein intake in the diet was investigated. A normal subject was given a diet adequate in calories and calculated to contain, respectively, 150, 75, and 0 grams of protein daily for each of three 8-day periods. It was observed that increases in the quantity of protein ingested only moderately increased the excretion of amino acids and of alpha amino nitrogen above that on the protein-free diet. No significant decrease in the amino acid excretion occurred during the protein-free period.

The excretion of "peptides" in the urine (determined as alpha amino nitrogen after hydrolysis of the urine) continued throughout the protein-starvation regime and represented about four times the excretion of "free" amino acids—a proportion of "peptides" to "free" amino acids similar to that excreted by this subject while receiving the diets containing 75 grams and 150 grams of protein.

The excretion in the urine of "free" amino acids and of "peptides" which occurred while no protein was fed must be considered endogenous in origin. This "minimum" excretion was increased by the ingestion of protein, although large fluctuations in the quantity of protein eaten resulted in only small changes in the quantity of amino acids excreted.

Excretion of "Peptides" Following Protein Hydrolysate Infusions. A normal subject given 75 grams of a partial protein hydrolysate ("Amigen") intravenously daily for four days as the sole source of nitrogen maintained weight and nitrogen balance. Of the nitrogen excreted daily during this period, 0.28 gram was "free" alpha amino nitrogen and 1.3 grams were "polypeptide nitrogen" determined as alpha amino nitrogen after hydrolysis of the urine. Since two-thirds of the total amino acids in this partial hydrolysate were "free" and one-third were "conjugated", the amount of polypeptide nitrogen excreted daily represented approximately 45 per cent of the polypeptide alpha amino nitrogen administered, while less than 5 per cent of the free alpha amino nitrogen given was lost. These observations confirm those of other investigators,[8] who further noted that, in contrast to the large loss by excretion in the urine of the peptides of "Amigen", those present in a partial hydrolysate of fibrin ("Aminosol") were lost to a much smaller extent.[9] Thus, there is considerable variation in the renal excretion of the polypeptides derived from different protein sources. However, following the infusion of a complete protein hydrolysate free of peptides (Merck*), no increase was observed in this laboratory in the excretion of "peptides" above that excreted on the control regime. Since "free" amino acids are lost to a smaller extent in the urine than are "peptides" following their intravenous infusion, a completely hydrolyzed protein would appear to be of greater nutritional value than a partially hydrolyzed protein.

Excretion of Amino Acids by Normal Subjects Following Amino Acid Infusions. Five hundred cc. of a 10 per cent solution of amino acids*[10, 11] (a complete acid hydrolysate of casein supplemented with tryptophane and containing 50 grams of amino acids) was infused without glucose to eight normal subjects while fasting at three rates of administration: approximately 2 mg., 6 mg., and 10 mg. of nitrogen per kilogram of body weight per minute, representing infusion times of approximately 1 hour, 20 minutes, and 10 minutes, respectively. The solution em-

* "Solution of Amino Acids Mixture, 10 per cent", developed and supplied by Merck and Company, Inc., Rahway, New Jersey. This product was used for all infusions reported in this paper unless otherwise indicated.

ployed was a complete acid hydrolysate of casein supplemented with tryptophane and glycine, and essentially devoid of glutamic and aspartic acids. Each 500 cc. infusion supplied 47 grams of amino acids, 7.5 grams of total nitrogen, 6.0 grams of alpha amino nitrogen, and the following amounts (*l*-form) of the "essential" amino acids: 2.5 grams arginine, 1.6 grams histidine, 3.8 grams isoleucine, 7.8 grams leucine, 4.2 grams lysine, 3.2 grams methionine, 2.7 grams phenylalanine, 1.1 grams threonine, 0.45 grams tryptophane, and 3.1 grams valine. The content of the individual amino acids in the mixture infused was determined by microbiological assay.[1]

The blood alpha amino nitrogen values (gasometric ninhydrin method of Hamilton and Van Slyke[12]) averaged 3.6 mg. per 100 cc. of plasma initially, rose to 22.5 mg. per cent 5 minutes after the infusion, rapidly fell to 7.2 mg. per cent 1 hour later, and returned to within normal limits by 4 hours, although most values were slightly above the pre-injection level and averaged 3.9 mg. per cent.

The excess excretion in the urine of alpha amino nitrogen above the control (*ad libitum* diet) occurred within the first 4 hours following the infusion, averaged 9.3 per cent of that infused, and coincided with the return of the blood amino nitrogen to within normal limits. The loss of amino nitrogen in the urine increased little with more rapid infusions (TABLE 2). Two additional subjects, in whom the urine was collected more frequently in the post-infusion period, excreted approximately 90 per cent of the amino nitrogen lost within the first hour after the infusion was completed. During this period, the most rapid decline occurred in the blood amino nitrogen.

The excretion above the control of each of the 10 individual amino acids administered likewise occurred within 4 hours following the infusion, and none exhibited a delayed excretion over the next 20 hours. These values are shown in TABLE 2. From 0.2 to 14.1 per cent of the individual amino acids infused was excreted in the urine at the slow rate of infusion. Thus,

TABLE 2

RELATION BETWEEN RATE OF INFUSION AND QUANTITY OF AMINO ACIDS EXCRETED IN THE URINE IN THE 24 HOURS AFTER ADMINISTRATION OF 500 CC. OF THE 10% SOLUTION OF AMINO ACIDS TO 8 NORMAL ADULTS

	Slow rate (2 infusions)		Moderate rate (3 infusions)		Rapid rate (3 infusions)		Average (8 infusions)	
	mg.*	%**	mg.*	%**	mg.*	%**	mg.*	%**
Arginine	8.7	0.3	18.1	0.7	37.8	1.5	23.0	0.9
Histidine	206	12.9	190	11.9	231	14.4	209	13.1
Isoleucine	9.1	0.2	55.6	1.5	98.6	2.6	61.0	1.6
Leucine	45.7	0.6	172	2.2	281	3.6	182	2.3
Lysine	149	3.5	264	6.3	354	8.4	268	6.4
Methionine	34.4	1.1	88.7	2.8	169	5.3	105	3.3
Phenylalanine	43.1	1.6	112	4.2	175	6.5	119	4.4
Threonine	155	14.1	175	15.9	196	17.8	179	16.3
Tryptophane	22.7	5.7	25.7	6.4	33.8	8.4	28.0	7.0
Valine	42.1	1.4	105	3.4	196	6.3	124	4.0
Total 10 "essential" amino acids	716	2.3	1206	4.0	1772	5.8	1298	4.3
"Free" alpha amino nitrogen	469	7.8	556	9.3	645	10.7	568	9.5

* Mg. of amino acid or of alpha amino nitrogen in excess of that excreted while receiving the *ad libitum* diet (TABLE 1).

** Per cent of administered amino acid or of administered alpha amino nitrogen excreted in urine.

the subjects excreted fairly large percentages of administered threonine and histidine, intermediate percentages of lysine and tryptophane, and smaller percentages of the other amino acids. In contrast to the marked variation in the excretion loss of individual amino acids at the slow rate of infusion, their additional loss with more rapid infusions varied little, so that an almost equivalent additional percentage of each amino acid given was excreted.

The patterns of the 10 "essential" amino acids administered intravenously, excreted in the urine, and (by difference) retained in the body are shown in TABLE 3. The proportion of the "essential" amino acids in the mixture differed considerably from that excreted in the urine. Thus, for example, although arginine and histidine were present in similar proportions in the mixture infused (8.1 and 5.1 per cent of the total 10 "essential" amino acids, respectively), their pattern in the urine was altered so that proportionately much more of the 10 amino acids excreted was composed of histidine (16.1 per cent) than of arginine (1.8 per cent). Since only a small portion of the infused amino acids was lost in the urine (averaging 4.3 per cent for all 10 amino acids assayed), the pattern of the amino acids retained in the body resembled quite closely the pattern administered.

It is not possible to ascertain why each of the amino acids was not excreted in the urine in the same proportion as administered (TABLE 3). At least three explanations exist: (1) differences in the quantity of amino acids infused as compared to their requirements by the body; (2) differences in the renal clearance of amino acids; and (3) differences in the rate of removal of amino acids from the blood stream by tissues. With each increase in the rate of infusion, however, the additional excretion of all the "essential" amino acids more closely resembled the composition of the amino acids infused.

The "essential" amino acids in the infusion mixture were preferentially retained by the subjects, while the dispensable amino acids were more freely excreted in the urine. The greatest retention was observed at the slowest rate of infusion. Thus, although the 10 "essential" amino acids comprised two-thirds of the total amino acids infused, they contributed less

TABLE 3

COMPARISON OF THE PATTERNS OF THE 10 "ESSENTIAL" AMINO ACIDS ADMINISTERED, EXCRETED, AND RETAINED FOLLOWING THE INFUSION OF 500 CC. OF 10% SOLUTION OF AMINO ACIDS TO 8 NORMAL ADULTS

	Per cent of total of 10 "essential" amino acids		
	Given*	Excreted**	Retained†
Arginine	8.1	1.8	8.4
Histidine	5.1	16.1	4.6
Isoleucine	12.6	4.7	12.9
Leucine	25.7	14.0	26.4
Lysine	13.9	20.6	13.6
Methionine	10.6	8.1	10.7
Phenylalanine	8.8	9.2	8.7
Threonine	3.5	13.8	3.0
Tryptophane	1.5	2.2	1.4
Valine	10.2	9.5	10.3
Total	100.0	100.0	100.0

* Calculated from the quantity of amino acids given.
** Calculated from the quantity of amino acids excreted, averaged value of 8 infusions, TABLE 2.
† Calculated from the quantity of amino acids retained (quantity administered minus quantity excreted).

than one-third of those excreted in the urine. The 10 "essential" amino acids contributed approximately one-fourth of the total amino acids excreted by these 8 normal subjects eating *ad libitum* and by the normal subject consuming varied amounts of protein.

Excretion of Amino Acids by Patients with Liver Disease. The average daily excretion of the "essential" amino acids in the urine of seven patients with severe liver disease (5 cirrhosis, 1 hemochromatosis, and 1 subacute yellow atrophy) eating *ad libitum* did not differ markedly from normal subjects (TABLE 1). In this small series, however, there was considerable variation among patients in the quantity of individual amino acids excreted. Four additional patients with active cirrhosis of the liver received 50 to 100 grams of the amino acid solution as the sole source of nitrogen intravenously daily for from 2 to 4 weeks. Positive nitrogen balance, maintenance of weight, decline in serum bilirubin, and clinical improvement occurred. The amino acid mixture was well tolerated. The loss of alpha amino nitrogen in the urine by the patients with liver disease was similar to that observed in normals following infusions of equivalent quantities of the hydrolysate. Furthermore, the post-infusion pattern of the "essential" amino acids excreted in the urine of the patients with cirrhosis was similar to that noted in normals. Hyperaminoacidemia and azotemia were not observed, nor was there a progressive increase in the urinary excretion of amino nitrogen. Previous studies in this laboratory[13] likewise demonstrated that the rate of removal of amino acids from the blood following the parenteral injection of a protein hydrolysate ("Parenamine") in patients with severe cirrhosis of the liver was little altered from that observed in normal subjects. It is concluded that intravenously administered amino acids are well tolerated by patients with severe liver disease, are metabolized and excreted in a manner differing little from normal, and may promote clinical improvement when administered as the sole source of nitrogen.

Excretion of Amino Acids in Wilson's Disease (Hepato-Lenticular Degeneration). Each of 5 patients with Wilson's disease eating *ad libitum* excreted greater quantities both of alpha amino nitrogen and of each of the "essential" amino acids in the daily urine than did normal subjects (TABLE 1).* The increased loss of amino acids in these patients was largely accounted for by an excessive rate of excretion during the fasting state. Thus, the hourly rate of excretion of alpha amino nitrogen in the fasting urines of patients with Wilson's disease averaged approximately 20 mg., in contrast to values of less than 5 mg. per hour for normal subjects. Although the patients' fasting plasma alpha amino nitrogen values were frequently slightly elevated, there was a lack of correlation between the level of amino acids in the blood and the increased excretion of amino acids in the urine which was always abnormal. Neither the oral ingestion of protein nor the intravenous infusion of the amino acid solution resulted in a greater excretion than observed for normals. The associated metabolic abnormalities observed in the Fanconi syndrome[15] were not present, nor did the liver disease appear to be responsible for the increased aminoaciduria. It is tentatively concluded that a renal mechanism of undetermined type is operative in Wilson's disease which allows increased spillage of amino acids in the fasting state but which normally accommodates increased loads of amino acids to the kidneys, as following the ingestion of food or the infusion of amino acids.

Oral and Parenteral Amino Acid Requirements. Minimal oral requirements of the "essential" amino acids will undoubtedly be found less than the paren-

* The finding of an increased excretion of amino acids in the urine of patients with Wilson's disease has been reported by others.[14]

teral requirements for several reasons. First, there is an increased excretion of amino acids when the parenteral rather than the oral route of administration is employed. Second, large single infusions of protein hydrolysates may be less efficient than divided oral feedings. Finally, it is more difficult to provide adequate carbohydrate to achieve maximum retention of protein by the parenteral route. Thus, it was observed that a protein hydrolysate which contained minimum amounts of phenylalanine maintained nitrogen equilibrium when administered orally, but was incapable of sustaining nitrogen balance when given parenterally until additional phenylalanine was provided.[7] The urinary loss of phenylalanine was greater both in absolute amount and in percentage of that administered when less than the minimum requirement of the amino acid was given and negative nitrogen balance resulted, than when sufficient of the amino acid was available for nitrogen equilibrium. The parenteral requirements for the other "essential" amino acids, also, are likely to be found greater than their oral requirements.

Practical Considerations of Protein Hydrolysate Therapy. The renal loss of amino nitrogen following the parenteral infusion of amino acids may be as great as 28 times the loss from the administration of an equivalent amount of nitrogen as whole protein orally.[7] Such losses following intravenous infusion as compared to oral feeding appear great until it is recalled that, with the excretion of 0.4 per cent (0.2 grams protein) of 50 grams of whole protein given orally or 10 per cent (5 grams protein) of 50 grams of hydrolyzed protein injected intravenously, the body retains at least 45 grams of the protein administered. Although it was observed that the renal excretion of amino acids was greater when 100 grams rather than 50 grams of amino acids was administered intravenously, the largest quantity of amino acids lost was so small in terms of grams of protein that the body retained for metabolic purposes more than 85 per cent of the protein given. Thus, the administration of protein or amino acids orally or intravenously, slowly or rapidly, and in large or small quantity was followed by excretion in the urine of but a small portion of the amino acids given. The provision of an excess of the "essential" amino acids in a hydrolysate should compensate for their increased excretion in the urine when the parenteral rather than the oral route is employed.

The patient to whom protein hydrolysates are being administered is concerned more with the unpleasant side effects and the duration of the infusion than with his urinary excretion of amino acids. Because of the length of time often required to infuse 50 to 100 grams of amino acids and the frequency of nausea and vomiting, parenteral protein hydrolysate therapy has unfortunately been limited, because of both inconvenience and discomfort to the patient, and the constant vigilance required by the physician. Since these disadvantages were minimized by the complete protein hydrolysate with which the studies reported here were conducted,[16] parenteral protein hydrolysate therapy may become more practical and the physician may, if necessary, provide optimum feeding of protein entirely by the intravenous route.

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Note. The Section of Psychology held a meeting on May 17, 1948, at which Professor O. H. Mowrer, Psycho-Educational Clinic, Harvard University, Cambridge, Massachusetts, presented a paper entitled, *Some Studies in the Psychology of Identification.* This will be published in Volume 11 of TRANSACTIONS.

SECTION OF ANTHROPOLOGY

PRELIMINARY REPORT ON A TRIP TO ETHIOPIA

By WOLF LESLAU*

The Ethiopic language aroused the interest of Western scholars as early as the 16th century. Old Ethiopic or Geez, at present the liturgical language of the country, and Amharic, the national language, were investigated first. Little by little, the other Semitic languages as well as the Cushitic and Nilotic linguistic families used in Ethiopia were studied also. Semitic scholars were soon aware, though not sufficiently, of the importance of Ethiopic for the understanding of the Semitic languages. Of the eight Semitic languages—Geez (old Ethiopic), Tigre, Tigrigna, Amharic, Harari, Gurage, Argobba, and Gafat—some are well enough known, others insufficiently, still others are known only by name.

It was with the intention of filling out the gaps in our knowledge of Ethiopic and of gaining an insight into the structure of the whole group that I went to Ethiopia in October, 1946, and stayed there until July, 1947. The trip was made possible through a Fellowship of the Guggenheim Foundation, a grant by the Viking Fund and another by Mrs. Denyse Mosseri-Dreyfus. I take this opportunity to express my sincere thanks to these sponsoring institutions and individuals.

Anyone who intends to do research work in Ethiopia will have to start with Addis Ababa, the capital. There he will get acquainted with the central government and obtain its permission to travel around the country. It is the Ministry of Pen which issues instructions to the various Ministries, and particularly to the Ministry of Interior, to facilitate the trip of the research worker. I personally had the privilege of having been received by the Emperor to whom, in a fifteen-minute conversation held in French, I explained the purpose of my visit to his country. He showed great interest in my project of surveying the languages of Ethiopia and gave me his support in traveling through all the regions in which I was interested. As from him, I received everywhere the fullest and most cordial cooperation.

Living conditions in Addis Ababa are pleasant. Several hotels accommodate the traveler with room and board. The currency used in Ethiopia is the Ethiopian dollar (*berr*), its exchange being 40 American cents. In this connection, it is interesting to note that half a dollar is called a shilling. To give an idea of the cost of living for a traveler, I should like to mention that, in 1946-47, the price of board at the Imperial Hotel varied between 10-12 Ethiopian dollars, that is, $4-$5 a day. For a traveler intending to spend only a limited time in Addis Ababa, it would not pay to rent a house, for the maintenance of which a certain number of servants would be necessary.

The research worker, and especially the student of history, religion, and languages should also avail himself, in Addis Ababa, of the help of the Ethiopian scholars—some of them of traditional standards, others who, through Western contacts, have a critical approach to science—who will readily give information on problems of traditional history, religion, and literature of the country. The National Library, although it contains a limited number of manuscripts, and the Museum attached to it will be helpful for the study of things Ethiopian.

As for the anthropologist, the city itself with its houses, its markets, and its people will be his best teacher. He will be especially interested to investigate the impact of Western civilization on Ethiopia. He will notice the general trend toward modernization, but he will still see side by side men and women clad in the traditional dress called *shamma* or toga, and others dressed

* Asia Institute, New York, N. Y. This paper, illustrated by lantern slides and recordings, was presented at the meeting of the Section on May 24, 1948.

in a perfect Western style; local courts held in the streets and government courts held in modern buildings applying Western codes; church schools with their teaching of Old Ethiopic only, and government schools where thousands of boys and girls receive modern education; mules on the sidewalks, on one hand, and two-wheel carriages called *gari*, cars, buses and airplanes on the other hand; religious festivals of local character, and others where the foreign representatives are invited; he will still hear the old Ethiopic songs at weddings and in coffee-houses, and modern Amharic songs with Western influence over the radio and at the weekly representations of the Patriotic Association.

The linguist will be able to find, in Addis Ababa, linguistic informants of nearly all the regions of the country, but his work will be most productive if he goes to the particular region in the language of which he is interested. This will enable him to make anthropological investigations at the same time.

After having spent six weeks in Addis Ababa, I went to the region of Gurage, a province situated southwest of Addis

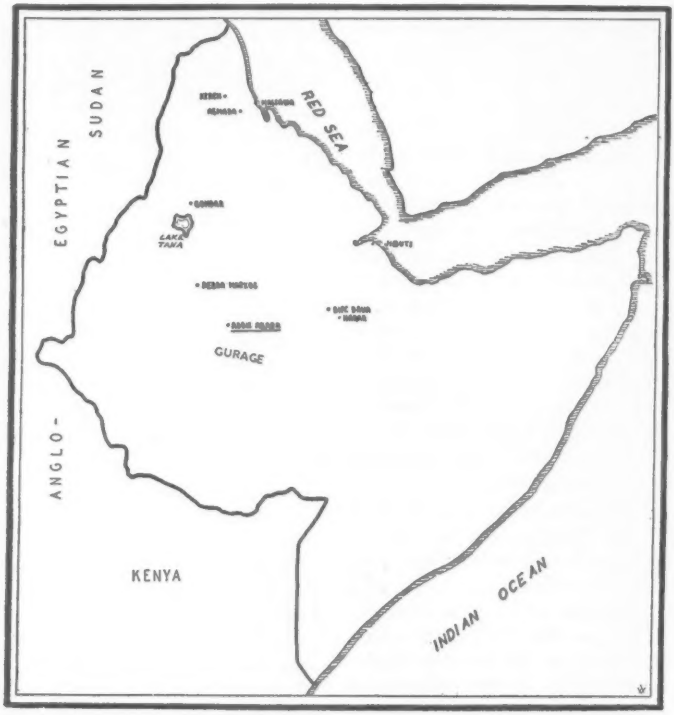


FIGURE 1.

Ababa (see FIGURE 1). The trip first took me to Wolqitte (over 90 miles on the Addis Ababa-Djimma road), and then a mule-ride of seven hours brought me to the town of Endeber, the capital of the tribe of Chaha. The mules and guides were put at my disposal by the local authorities of Wolqitte.

The traveler will easily recognize the province of Gurage by the clean, round houses and by the characteristic banana-like plant called *ensat* in Amharic, generally known under the name of *musa ensete*. This plant provides the Gurage with flour; he makes cord out of the fiber, and the leaves of the plant also have various usages.

A few words on the country of Gurage, its people and languages might be useful to show the rich soil this province represents for the research worker.

Gurage is a relatively small province, and it is, therefore, all the more surprising to observe the great diversity of tribes, religions, and languages. It seems probable that the original population of the present-day Gurage was a Sidamo group from the South. The country was then occupied and settled by military colonies from the North of Ethiopia. Still other parts of the population might have come from the region of Harar. This situation is reflected in the ethnic, religious, and linguistic status of Gurage. In fact, there is a great variety of tribes, each of them divided into sub-tribes and families. The most important tribe seems to be that of Chaha. Concerning religion, the Gurage are generally Moslems, pagans, and Ethiopian-Christians. Some of them are Roman Catholics. As for the languages and dialects, their variety is as great as that of the tribes. We count about 5-6 main groups, each of them with a variety of dialects.

Endeber is the administrative chief-town of the Chaha section. It is inhabited by Gurage and Amharas. I was the guest of the native Catholic Mission there, which is situated at the outskirts of the town. The Mission has two sections, for boys and for girls. For my linguistic work, I had at my disposal boys from the age of ten to twenty-five living in the Mission, all of whom spoke Amharic beside their native tongue, while some of them knew French or English. My work consisted in collecting texts of anthropological and folkloristic character, material for a grammar of the dialect of Chaha, and a vocabulary of seven different dialects. I also had occasion to assist at religious festivals, and to travel around the country to observe the people at their work and leisure. Very often, I encountered young boys and girls who, eager to study, every day walk as much as three to four hours both ways to attend school at Endeber.

At this point, I should like to say a word about the food situation in the interior of Ethiopia as it concerns the traveler. If he can get used to pepper, which is the basic condiment of every kind of Ethiopian food, he will have no difficulty at all. Otherwise, he will have to carry food with him and have it cooked in his own way. I, myself, lived on chicken twice a day for seven weeks, besides eating the pancake-like native bread. The traveler will, of course, be careful to boil the water he uses.

After a stay of seven weeks in Endeber I fell ill and had to be carried down on a bed for seven hours to Wolqitte. From there, a car took me back to Addis Ababa. The illness was caused by fatigue. After a rest of two weeks in Addis Ababa, I went east to Harar, in order to investigate the language called *adare*.

The traveler can go to Harar either by train or by plane to Dire-Daua (265 miles), from where a bus will take him in about two hours (33 miles) to Harar. At the approach of the city, he will be agreeably surprised at the sight of a great variety of fruit and vegetables, such as bananas, oranges, lemons, papayas, and tomatoes.

Dire-Daua is the main rail center of the Djibouti-Addis Ababa line. The city has a European quarter occupied by French, Italians, Greeks and Armenians, besides the native quarter. The main body of the native population are Somalis and Gallas; the mosques of the city bring proof of the Moslem faith of its inhabitants. Since I had no particular linguistic interest in Dire-Daua, I spent only two days in the city, making recordings of Somali and Galla music.

The city of Harar presents a particular interest from a historical, anthropological, and linguistic point of view. Old Harar is a walled city with five gates. A little over an hour is sufficient to walk all around the walls. Even before the Italian occupation there were some buildings, governmental and private, outside the walls of the Old City. The Italians chose Harar as capital of the whole province and considerably extended this new section. The traveler who intends to spend some time in Harar will easily find a convenient house to rent, but he will welcome the good living conditions at the Grand Hotel.

Harar has attracted many students of history and anthropology, such as Paulitschke and Burton, and for good reasons. It was the capital of the Moslem-Ethiopian state and is still the center of Islamic culture in Ethiopia. Its inhabitants are mainly Gallas and Somalis of Moslem faith, but there are also Amharas. The student of religion and anthropology will be interested in studying the Arab life and culture in the surroundings of Gallas, Somalis, and Amharas. Everything indicates that Moslems live in the city: the seventy mosques, the bazars, the veiled women, the turbans of the men, the use of the narcotic plant (called *qat* in Arabic) in the morning hours, the particular style of the houses with nooks in the walls, the carpets, and the basket work. A city life of the Middle Ages can still be observed inside the walls of the city, bare of all vegetation. Arabic, Somali, Galla, Amharic, and the language particular to Harar called *adare* are spoken inside the walls, and very often by the same person.

I was particularly interested in the *adare* language. This is a Semitic language spoken only inside the walls, and with the growth of Amharic influence through the schools and the administration it might disappear. In Harar, I checked the linguistic material known through the various works, and collected new material for a grammar, a vocabulary of about 2,500 roots, texts in prose, and songs in Ancient Harari. The student of Harari will still find manuscripts of that language written in Arabic characters. I myself acquired a collection of songs in Ancient Harari in honor of the prophet Mohammed.

After a stay of six weeks in Harar, I returned to Addis Ababa to prepare another trip to Gojjam and Begemder, both north of Addis Ababa. The traveler can, of course, still go by mule to any of these regions, but he will be only too glad to go either by truck crossing the Blue Nile or by plane to Debra Markos, the capital of Gojjam (160 miles from Addis Ababa). He will reach Debra Markos by truck in approximately two days, by plane in forty minutes.

Ethiopian life can best be observed in the region of Gojjam. It is true that one notices here, too, the products of modernization—the airplane, the school, and the administration contribute their share to it—but the Ethiopian of Gojjam, and for that matter of Gondar, is still resistant to things which are not Ethiopic. Debra Markos is a perfect example of what was the ancient Ethiopian city. The palace of the Governor dominates the city, and the main means of transportation is the mule. The regional chiefs come from their provinces accompanied by their servants. The national feast, called *geber,* is still given in the traditional way, and at the end of it one will hear the noted *fukkara* or boasting of the warrior emitted in a particular modulation.

But Gojjam is more than all this. It is intimately linked with the whole religious and political history of Ethiopia. My interest in Gojjam was of a particular nature. In the region of the Blue Nile, there was a population called Gafat. This population had a language of its own, a Semitic language which was still understood, if not spoken, at the end of the 18th century. At that time, James Bruce had the Song of Solomon translated into various Ethiopic languages, including Gafat. All attempts by recent travelers to discover whether Gafat was still spoken remained in vain.

In fact, the inhabitants of Gafat at present speak Amharic, the national language of Ethiopia. On the basis of the only document on Gafat preserved in the Bodleian Library at Oxford, I worked out the structure of the language and published it, in 1945, together with the Gafat text and translation, in the *Gafat Documents*. Intrigued by a remark of the Abyssinian historian, Aleqa Tayye, in his *History of Abyssinia*, published in Amharic in 1927, that the inhabitants of Gafat still use their own language when they are among themselves, I tried to find out whether this language was still in existence. Dedjazmach Kebbede, the governor of Gojjam, took interest in my investigation. Not being a native of Gojjam, he was unable to give me information on the subject, but made an inquiry among the elders of the region. This investigation brought me to Burie, 60 miles north of Debra Markos.

The reader will understand my disappointment when, instead of the expected Gafat, I came across an artificial language which consisted in inverting the syllables, like saying *teb* instead of *bet*. Also, I was told that some judges in a native court knew Gafat, but when I came to see them I found out that, instead, they were interested in Gafat and wanted to study it from my *Gafat Documents*. Finally, after two weeks of inquiry, I found four people (three men and a woman, all of them old), from the region of Womberma in the vicinity of the Blue Nile who still spoke Gafat. I took them with me to Debra Markos, since the region of the Blue Nile is lowland and, consequently, very hot, and was able to investigate their language.

The linguist will be interested to know that, in my inquiry on Gafat, I came across special languages, such as the language of the merchants, the language of the chanters, and that of those possessed by the spirits. All these people speak Amharic, but use, for professional purposes, a more or less artificial language.

I left Gojjam after a stay of four weeks and went by plane to Gondar, the capital of Begemder. Gondar is situated to the north of Gojjam at a distance of 200 miles from Debra Markos. If Harar is the center of Islamic culture in Ethiopia, Gondar was, and in some respects still is (at least as tradition goes) the center of Ethiopian culture proper. It was the capital of Ethiopia in the 17th century. The various castles of the city, and of other places around Gondar, such as Old Gorgora on the Lake Tana, bear witness of the Portuguese in Ethiopia, who, as is well known, were asked by the kings to deliver the country from the Arab invasion in the 16th century.

Gondar is the city of old tradition in learning and Ethiopian scholarship. With its province, it is the center of numerous churches and monasteries. It has still preserved the old style in liturgical chanting, and the entire province has probably the finest preserved Ethiopic manuscripts and old Ethiopian paintings. Gondar is the most appropriate place for research in traditional history, liturgy, and religious problems. It is true that Ethiopian scholars are attracted by Addis Ababa, and Gondar is losing its value as a cultural center, but treasures of tradition still lie hidden in the churches and monasteries, and the priests and monks still can reveal much information on the ancient history of Ethiopia.

The student of linguistics will have an opportunity to examine the dialectal differences between the Amharic of Gondar and that of Shoa, and, besides, he will find himself in the center of the Cushitic languages of Agau. The student of things Ethiopian will not limit himself to a stay in Gondar, but will travel in the whole of the province of Begemder to see its monasteries and churches, and will in particular go for a boat ride on Lake Tana, the description of which would go far beyond this report. He will be rewarded not only by the literary treasures and relics of the ancient Ethiopians, but also by the sight of the green mountains, the deep valleys, and the numerous attractive islands of Lake Tana.

My main interest in Begemder lay in the investigation of the problem of the Falashas, that is, the Ethiopians of Jewish faith. The existence of Jews in Ethiopia was brought to the attention of the Western world as early as the 12th century. Beginning with the 18th century, various travelers visited the Falashas, but most of them had a missionary tendency which appreciably diminishes the usefulness and objectivity of their reports, so that I decided to concentrate my special attention on the Falashas. I left for Uzaba, a province to the southeast of Gondar, entirely inhabited by Falashas, and lived there for about three weeks, in the village of Ambworo.

I arrived at Passover—it was in the month of April—and, consequently, no one worked in the whole village. We gathered every day in the synagogue, priests, elders, and young men, and to the best of their knowledge they answered all my questions concerning their life. I visited all the neighboring villages and never came back from any of these visits without a goat, hens or eggs brought to me as gifts from all the Falasha huts. I also spent some days with the Falashas of the region of Seqelt to pay a visit to their monks. In this way, I gathered information on the most important phases of cultural, religious, and social life of the Falashas.

Not only did the Falashas receive me in the most cordial way I mentioned before, but nearly everywhere in Begemder the Ethiopians demonstrated their traditional way of hospitality. I shall never forget the reception in Gana Yohannes, in the region of Seqelt, where the governor of Seqelt came to receive me with fifty of his men and gave a copious *geber* in my honor.

In May, the small rains made their appearance and I went back to Addis Ababa. It is not an easy enterprise to travel in the interior during the rainy season. In some regions, it is even impossible to do it. I might add that, during the dry season which lasts from October to June-July, the climate of the highland is very pleasant: not too hot in the daytime, and cool at night. The same cannot be said of the lowland, where the heat is made more disagreeable by the presence of many flies. I intended to undertake a trip to the region of Ankober, to the north of Addis Ababa, to investigate Argobba, a language doomed to disappear. However, I felt the effects of fatigue and decided to work on Argobba with informants found in Addis Ababa. An Argobba Moslem woman, and occasionally a man, were my main informants, and with their help I gathered enough material for an analysis of the language.

In all the regions I visited, I made recordings of music, songs, and text specimens.

Before leaving Addis Ababa, I had the privilege to be received once more by the Emperor. This time, the conversation was in Amharic.

I left Addis Ababa in June, by bus for Asmara. The distance from Addis Ababa to Asmara is a little over 600 miles. I could, of course, have traveled by plane, but the trip by bus gave me the opportunity of crossing the whole of Ethiopia, the province of Tigre, and a part of Eritrea. The general impression the traveler gains from the northern part of Tigre and Eritrea is different from the remaining part of Ethiopia: Eritrea has a desert appearance. As for Asmara, it has the aspect of a European city; it also has a native section.

Besides the Cushitic and Nilotic languages spoken in Eritrea, the Semitic languages of Tigrigna and Tigre are used. Tigrigna being relatively well known, I concentrated my special efforts on the investigation of Tigre. To do this, I established myself in the city of Keren, 58 miles northwest of Asmara. Keren is a pleasant town, of a very "Oriental" aspect. I checked there the material previously published on Tigre and was able to supplement our knowledge in the domain of the grammar and vocabulary. Beside my lin-

guistic work, I visited the various parts of the country, particularly Massawa, and also the monasteries of Debra Sina and of Debra Bizen, well known in the ecclesiastic history of Ethiopia.

Needless to say that the student of Ethiopia will be greatly interested in Eritrea if he is reminded of the fact that its population is half Christian, half Moslem; that the Italians lived in Eritrea for about fifty years and left there traces of Western culture; that ethnically the population is of mixed origin; that it has many interesting monasteries; that Semitic, Cushitic, and Nilotic languages are spoken there; and, finally, that the historical ties between Eritrea and the remaining part of Ethiopia are of the greatest importance for the future of the country.

The reader who knows something about Ethiopia will miss, in my description, many parts of the country not mentioned here. In fact, nothing or not enough was said about the Dankali, the Somali, the Galla, the Sidamo, the Agau populations, and others. The reason for not mentioning these provinces and populations is simply that, as I stated in the beginning, my interest for this trip was a survey of the Semitic languages of Ethiopia, and, consequently, I visited only those regions in which these languages are spoken.

If the student of Ethiopia keeps in mind the great amount of non-Semitic languages spoken there, the variety of populations and cultures, and, at the same time, the political unity of the country, he will be aware of the great interest Ethiopia offers to the student of history, religion, languages, anthropology, and the social sciences. These studies will throw light not only on Ethiopia, but also on Africa and on the present-day and ancient Near East. Whatever his particular field, the student will have satisfactory results in his investigation only if he has direct contact with the people. For keeping this up, he will need a speaking knowledge of Amharic, which is spoken and understood nearly all over Ethiopia. His work will be still more fruitful if he acquires a knowledge of the religious, cultural, and political past of the country.

In concluding, I should like to reiterate my thanks to the already mentioned institutions and individuals who made this trip possible; to the American officials, Mr. Felix Cole, formerly Minister of the United States to Ethiopia, and Mr. William H. Beach, Consul General, who manifested great interest and understanding for scientific work; to Prince Makonnen, Duke of Harar; to the many Ethiopian officials, and particularly to His Excellency Ato Aklilou Habte Wold, Vice-Minister of Foreign Affairs; Ato Akale Worq, Director of the Ministry of Education; Blatta Ayyele Gebre, Vice-Governor of Harar; Dedjazmach Kebbede, Governor of Gojjam; the Princess Tanagne Worq; and Bitwaddad Andarge, son-in-law of the Emperor and Governor of Begemder. Their cordial cooperation was extremely helpful in accomplishing my task. I also want to thank my many Ethiopian friends as well as the man in the street who helped me to feel and to understand Ethiopia. My gratitude goes particularly to His Majesty, the Emperor Haile Selassie, whose sincere interest and approval of my project provided me with the willing cooperation of the Ethiopian people.

SECTION OF PHYSICS AND CHEMISTRY

COPOLYMERIZATION REACTIONS

By TURNER ALFREY, JR.*

Introduction. The tendency of many unsaturated organic compounds to form chain polymers is well known. While this polymerization reaction is by no means limited to vinyl compounds (vinylidene compounds, dienes, etc., also polymerize), the term "vinyl polymerization" will be used as a generic expression in certain parts of the discussion. When more than one monomeric material is present, the reaction is called "copolymerization," and the resulting product is called a "copolymer."

Vinyl polymerization is a chain reaction consisting of at least three steps: (1) chain initiation or activation; (2) propagation, or growth; and (3) chain termination. In the cases of thermal and peroxide-catalyzed polymerization, the activation step involves the formation of a free radical, and the propagation step consists of the addition of a monomer molecule to the growing chain without loss of the free radical activity.

$$\sim\!\sim CH_2\overset{\overset{H}{|}}{\underset{X}{C}}\bullet + CH_2 = CHX$$

$$\longrightarrow \sim\!\sim CH_2CHXCH_2\overset{\overset{H}{|}}{\underset{X}{C}}\bullet$$

On the other hand, when catalysts such as BF_3 are used, the reaction proceeds by an ionic mechanism. The latter type of polymerization will not be explicitly considered here, although many of the generalizations developed here should apply to ionic as well as free radical polymerization.

The complete kinetic analysis of a vinyl polymerization reaction is rather involved. There are a number of different possible mechanisms for both the activation and the termination steps. Furthermore, such additional processes as chain transfer take place under certain conditions. If more than one monomer is participating in the reaction, the problem obviously becomes still more difficult.

Fortunately, a considerable amount of information concerning copolymerization can be derived from consideration only of the *propagation* step of the reaction. The *rate* of copolymerization, and the molecular weight distribution of the resulting copolymer, depend, of course, upon the rates of initiation and termination as well as of propagation. The *chemical composition* of the copolymer, however, is determined almost entirely by the competition among the various propagation reactions. From a practical standpoint, this means that there is a simple equation (the "copolymer composition equation") which relates the composition of a copolymer to that of the monomer mixture from which it is formed. From a more fundamental standpoint, this relationship makes possible the quantitative study of the propagation step of polymerization.

The Copolymer Composition Equation:

$$\frac{dM_1}{dM_2} = \frac{M_1}{M_2}\cdot\frac{r_1M_1 + M_2}{r_2M_2 + M_1}$$

Consider the two vinyl monomers M_1 and M_2. Four propagation rate constants govern the growth of active polymer chains. These four reactions are summarized in TABLE 1. The symbols M_1* and M_2* refer to the concentration of active chain ends of the types M_1 and M_2.

In the steady state of copolymerization, $k_{12}M_1{}^*M_2 = k_{21}M_2{}^*M_1$. This indicates that, in the steady state, the rate of reaction of polymers ending in M_1 with monomer M_2 must be equal to the rate of reaction of polymers ending in M_2 with

* Polytechnic Institute of Brooklyn, Brooklyn, N. Y. This paper was presented at the meeting of the Section on February 3, 1948.

The Section of Physics and Chemistry held a meeting on May 4, 1948, at which Professor Edward Saibel, College of Engineering and Science, Carnegie Institute of Technology, Pittsburgh, Pennsylvania, presented a paper entitled, *Applications of Chemical Reaction Rate Theory to Mechanical Phenomena,* which was illustrated. No abstract of this has been received.

TABLE 1

Growing chain	Monomer adding	Rate of process	Reaction product
$\text{——}M_1^*$	M_1	$k_1(M_1^*)(M_1)$	$\text{——}M_1\text{-}M_1^*$
	M_2	$k_{12}(M_1^*)(M_2)$	$\text{——}M_1\text{-}M_2^*$
$\text{——}M_2^*$	M_1	$k_{21}\cdot(M_2^*)(M_1)$	$\text{——}M_2\text{-}M_1^*$
	M_2	$k_{22}\cdot(M_2^*)(M_2)$	$\text{——}M_2\text{-}M_2^*$

monomer M_1. It is now possible to express M_2^* in terms of M_1^*, although this approach cannot yield absolute values for M_1^* and M_2^*:

$$(M_2^*) = \left(\frac{k_{21}}{k_{12}}\right)\left(\frac{M_2}{M_1}\right)(M_1^*)$$

The rate of consumption of monomer M_1 is given by EQUATION 2:

$$-\frac{dM_1}{dt} = k_{11}(M_1^*)M_1 + k_{21}(M_2^*)M_1$$

Similarly, the rate of addition of M_2 to the polymer is

$$-\frac{dM_2}{dt} = k_{12}(M_1^*)M_2 + k_{22}(M_2^*)M_2$$

Finally, the ratio between the rate of addition of M_2 and the rate of addition of M_1 is given by:

$$\frac{dM_1}{dM_2} = \frac{k_{11}(M_1^*)M_1 + k_{21}(M_2^*)M_1}{k_{12}(M_1^*)M_2 + k_{22}(M_2^*)M_2}$$

Let us introduce the new variables r_1 and r_2.

$$r_1 = k_{11}/k_{12}$$

$$r_2 = k_{22}/k_{21}$$

EQUATION 4 can be rewritten in terms of r_1 and r_2.

$$\frac{dM_1}{dM_2} = \frac{M_1}{M_2}\cdot\frac{r_1M_1 + M_2}{r_2M_2 + M_1}$$

The ratio of the rates of addition of the two monomers is, of course, also an expression for the ratio of those two monomers in the resulting polymer.

$$\frac{m_1}{m_2} = \frac{M_1}{M_2}\cdot\frac{r_1M_1 + M_2}{r_2M_2 + M_1}$$

It is clear that the initial polymer, in general, does not possess the same composition as the monomer mixture, but is relatively richer in one component than in the other. The more reactive monomer is thus used up more rapidly, leaving the monomer mixture relatively poorer in this compoment. It follows necessarily that the last polymer to be formed is relatively richer in the less reactive constituent. The overall polymerization product, thus, is a mixture of copolymer molecules of different chemical constitution.

Distribution of Monomer Sequences. EQUATION 1 gives the *overall* molar composition of the copolymer molecules which form in a given monomer mixture. It is possible, however, to deduce a much more detailed picture of the copolymer structure from the rate constant ratios r_1 and r_2. Let us designate each group of suc-

cessive M_1 units in the copolymer as a *sequence* of M_1 units. Each M_1 sequence is bounded on both ends by a sequence of M_2 units. The copolymer molecules consists of an alternating series of M_1 and M_2 sequences.

Depending on the relative values of the propagation rate constants, M_1 and M_2 sequences of varying sizes will be found in the polymer. When the rate constants k_{11} and k_{22} are large as compared to the rate constants k_{12} and k_{21}, one can expect to find long sequences of M_1's followed by long sequences of M_2's, whereas, if the relative values of those rate constants are reversed, then the polymer will contain short groups of M_1's followed by short groups of M_2's.

By defining four probabilities P_{11}, P_{12}, P_{21}, P_{22} for the probabilities of a monomer of the type M_1 adding to a chain ending in M_1*, a monomer of the type M_2 adding to a chain ending in M_1*, a mononer of the type M_1 adding to a chain ending in M_2* and a monomer of the type M_2 adding to a chain ending in M_2*, it is easy to calculate the distribution of lengths of M_1 and M_2 sequences.

To a chain ending in M_1*, only one of two things can happen. (1) a monomer M_1 or (2) a monomer M_2 can add; therefore, the probability P_{11} will be the rate of addition of M_1 over the rate of addition of M_1 + the rate of addition of M_2, etc.

$$P_{11} = \frac{k_{11} M_1}{k_{11} M_1 + k_{12} M_2} = \frac{r_1 M_1}{r_1 M_1 + M_2}$$

$$P_{12} = \frac{k_{12} M_2}{k_{11} M_1 + k_{12} M_2} = \frac{M_2}{r_1 M_1 + M_2}$$

$$P_{21} = \frac{k_{21} M_1}{k_{21} M_1 + k_{22} M_2} = \frac{M_1}{M_1 + r_2 M_2}$$

$$P_{22} = \frac{k_{22} M_2}{k_{21} M_1 + k_{22} M_2} = \frac{r_2 M_2}{M_1 + r_2 M_2}$$

The distribution of sequence lengths (n_1) of M_1's and (n_2) of M_2's can be determined by the following consideration: To the first constituent M_1 of a group of M_1's picked at random, the addition reaction k_{11} (M_1*) (M_1), whose probability is P_{11}, has had to be repeated (n-1) times, to yield a group of nM_1's. The probability of these (n-1) consecutive additions is $(P_{11})^{(n-1)}$. This sequence of reactions must then be followed by the addition of M_2, which has the probability P_{12}. The probability that a group of M_1's picked at random contains n_1 members is thus:

$$N(n_1) = P_{11}^{(n_1 - 1)} (1 - P_{11})$$

This is also the number distribution function for the lengths of M_1 sequences.

Similarly, the number distribution function for sequences of M_2's is given by:

$$N(n_2) = P_{22}^{(n_2 - 1)} (1 - P_{22})$$

where $N(n_2)$ represents the fraction of all M_2 sequences which possess n_2 members.

The average length of the M_1 sequences must, of course, bear a very simple relationship to the average length of the M_2 sequences, since there will be (neglecting end sequences) just as many M_1 sequences as M_2 sequences in the copolymer. If $\bar{n}_1$ and $\bar{n}_2$ are the number average lengths of M_1 and M_2 sequences, respectively, and M_1/M_2 is the number ratio of M_1's to M_2's in the molecule, then $\bar{n}_1$ and $\bar{n}_2$ are related by the expression:

$$\bar{n}_1 / \bar{n}_2 = M_1 / M_2$$

These M_1 and M_2 sequences are strung together in a *random order* in the copolymer molecule. In other words, the detailed structures of copolymer molecules formed from a given monomer mixture are those structures which would be formed if one took a container of M_1 sequences (distributed in length according to EQUA-

TION 9) and a container of M_2 sequences (distributed in length according to EQUATION 10), and alternatively picked out M_1 sequences (randomly) from one container and M_2 sequences (randomly) from the other container.

Speculations Regarding Effect of Structure on Reactivity with Free Radicals. In the previous section, it was demonstrated that the chemical composition and molecular structure of a polymer are determined by the competition of the two monomers for both types of free radicals (growing chain-ends). We now take up the important question: "How does the reactivity of a monomer in copolymerization depend upon its molecular structure?" The following generalizations can be made:

(1) Monomers which possess substituents on both carbon atoms of the C=C double bond (1,2-disubstituted ethylenes) exhibit a *specific reluctance* to add to *themselves* in the growing chain, although they may add to "vinyl" free radicals quite readily.

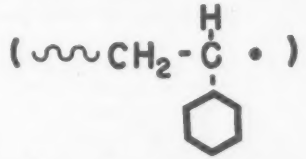
If, for example, M_1 represents styrene and M_2 represents diethyl maleate, the rate constant k_{22} is zero within experimental error, but the rate constants k_{11}, k_{12} and k_{21} all have finite values.

This is believed to be a *steric* effect.

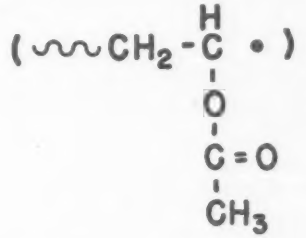
This leads to a number of interesting consequences. For example, these 1,2-disubstituted compounds exhibit practically no tendency to polymerize alone, but can often *copolymerize* readily with vinyl monomers. Maleic anhydride reacts with the styrene free radical about 20 times as fast as does styrene monomer (although maleic anhydride will not add to the maleic anhydride radical at all). Over a wide range of monomer ratios, the copolymer is almost exactly 50-50, with the structure -1-2-1-2-1-2-1-2-. This regular alternating structure represents the largest amount of M_2 which can be put into the copolymer, even with a large excess of M_2 in the monomer mixture.

(2) The chief factor governing the reactivity of a vinyl monomer is the amount of *resonance stabilization* of the *radical adduct* which is formed by the reaction of the monomer.

Thus, styrene exhibits a *strong* tendency to add to any given free radical, because the resulting adduct free radical is *stabilized by resonance:*



(This effect is identical with that which makes the triphenylmethyl radical so very stable.) Vinyl acetate, on the other hand, exhibits a *weaker* tendency to add to any given radical, because the resulting radical in this case is *not* stabilized by resonance:



When styrene and vinyl acetate must compete with each other for a given free radical, the styrene will be 30 to 50 times as reactive as the vinyl acetate.

(3) A second factor, of somewhat smaller importance, is the electrical *polarity* of the double bond. A substituent such as -CN, which is *m*-directing in the benzene ring, can be expected to *withdraw electrons* from the polarizable double bond, thus giving the double bond a + character. Similarly, such a group will give to a *free radical* a + polarity. Other substituents will be *electron-donating,* and will give a negative polarity to the double bond of the monomer and to

the carbon atom of the corresponding free radical. A free radical with a positive + polarity will exhibit a particular preference for a monomer with a negative double bond, and vice versa.

(4) A semi-quantitative scheme has been developed in which the *general reactivity* of a monomer (determined by the resonance effect) is denoted by the symbol Q, and the polarity is denoted by the symbol e. In this scheme, the copolymerization ratios are given by the equations:

$$r_1 = \frac{k_{11}}{k_{12}} = \left(\frac{Q_1}{Q_2}\right) e^{-e_1(e_1 - e_2)}$$

$$r_2 = \frac{k_{22}}{k_{21}} = \left(\frac{Q_2}{Q_1}\right) e^{-e_2(e_2 - e_1)}$$

This pattern of behavior arises from the hypothesis that the rate constant for the addition of a monomer of type 2 to a free radical of type 1 can be given to a good approximation by an expression such as the following:

$$k_{12} = P_1 Q_2 e^{-e_1 e_2}$$

P_1 is characteristic of radical 1; Q_2 is the mean reactivity of monomer 2; e_1 is proportional to the charge on the end group of radical 1; and e_2 is proportional to the charge on the double bond of monomer 2.

It would be a reasonable speculation, for example, to write:

$$k_{12} = P_1 Q_2 e^{-E_1 E_2 / rDkT}$$

where ε_1 and ε_2 are the actual charges on the radical 1 and monomer 2, D is the effective dielectric constant and r the distance of separation in the activated complex. The characteristic constant, e, would then be related to the actual charge, E, by the proportionality:

$$e_1 = E_1 / \sqrt{rDkT}$$

The relative rates at which monomers 1 and 2 compete for radicals of type 1 is thus given by an expression such as the following:

$$k_{11} / k_{12} = (Q_1 / Q_2) e^{-e_1(e_1 - e_2)}$$

$$k_{21} / k_{22} = (Q_1 / Q_2) e^{-e_2(e_1 - e_2)}$$

The constant P, connected with the reactivity of the radical, cancels out.

Consider, for example, the results reported by Lewis, Mayo and Hulse.[1] These authors investigated the copolymerization of all possible pairs among the four monomers: styrene, methyl methacrylate, acrylonitrile, vinylidene chloride. TABLE 2 gives their results for the relative reactivity of each monomer with each type of free radical.

TABLE 2

RELATIVE RATES OF MONOMER ADDITION

Monomer	Radical: Styrene	Acrylonitrile	Methylmethacrylate	Vinylidene chloride
Styrene	(1.0)	25.0	2.0	7.0
Acrylonitrile	2.5	(1.0)	0.8	2.7
Methyl methacrylate	2.0	7.0	(1.0)	4.1
Vinylidene chloride	0.5	1.1	0.4	(1.0)

These reactivities are consistent with the values for Q and e given in TABLE 3.

TABLE 3

Monomer	Q	e
Styrene	1.00	−1
Methyl methacrylate	0.64	0
Acrylonitrile	0.34	+1
Vinylidene chloride	0.16	0

This order of the four monomers fits in very well with the theoretical expectations based upon resonance stabilization as the principal factor influencing general monomer reactivity. Styrene, the most "reactive" of the set, exhibits the greatest number of resonance structures for the radical which is formed by addition to the chain. Methyl methacrylate comes second, acrylonitrile next, and vinylidene chloride last. In fact, no ordinary "conjugation" at all exists in the vinylidene chloride radical. A small amount of resonance stabilization might be expected from weakly contributing structures in which the carbon-chloride bond has a certain double-bond character analogous to that postulated in chlorobenzene.

The "*e*" values are consistent with the hypothesis that the double bond in styrene —and therefore the carbon atom with the odd electron in the styrene radical—is electron-rich (negative), the double bond in acrylonitrile is electron-poor (positive), while the double bonds in methyl methacrylate and vinylidene chloride are approximately "neutral". If this is true, one would expect the relative rate of addition of each neutral monomer to all radicals to be determined primarily by the overall reactivity of the monomer, and also for the competition of all monomers for the neutral radicals to be determined by the overall reactivities of the monomers. Furthermore, the rate of addition of styrene to styrene radical and acrylonitrile to acrylonitrile radical should be reduced by electrostatic repulsion to values smaller than those expected on the basis of the mean reactivities of the monomers; likewise, the rate of styrene adding to acrylonitrile, and *vice versa*, should be increased by electrostatic attraction beyond the values expected on the basis of mean monomer reactivities alone.

(5) It is now clear why the separate polymerization behavior of individual monomers is a poor guide to their copolymerization behavior. Styrene monomer is a *reactive* monomer, but the styrene-free radical is a stable, *unreactive radical.*

Vinyl acetate monomer is (relative to styrene) an *unreactive monomer,* but the vinyl acetate *free radical* is unstable and *reactive.*

These two effects tend to *compensate* one another. The self-growth of styrene (unreactive radical + reactive monomer) may be quite comparable in rate with the self-growth of vinyl acetate (reactive radical + unreactive monomer). But when vinyl acetate monomer and styrene monomer must compete for the *same* free radicals (as in copolymerization), the greater reactivity of the styrene is very evident.

REFERENCES

1. **Mayo, F. R., F. M. Lewis, & C. Walling.** 1948. J. Am. Chem. Soc. **70:** 1529.
2. **Alfrey, T., & C. C. Price.** 1948. J. Polymer Sci. **2:** 101.
3. **Walling, C., E. R. Briggs, K. B. Wolfstin, & F. R. Mayo.** 1948. J. Am. Chem. Soc. **70:** 1537.

CONFERENCES HELD

SECTION OF PSYCHOLOGY

CONFERENCE ON "HUMAN ENGINEERING": MAY 10 AND 11, 1948

The Section of Psychology held a Conference on "Human Engineering." Doctor Lawrence E. Abt, Research Division, College of Engineering, New York University, New York, N. Y., was the Conference Chairman in charge of the meeting.

The program consisted of the following papers:

Monday, May 10

Morning Session. Chairman, Jack W. Dunlap, Dunlap, Morris and Associates, Inc., New York, N. Y.

"Introductory Remarks," by the Conference Chairman.

"A Program of Human Engineering Research," by Leonard C. Mead, Special Devices Center, Office of Naval Research, Sands Point, L. I., N. Y.

"Handbooks of Human Engineering Data," by John L. Kennedy, Institute of Applied Experimental Psychology, Tufts College, Medford, Massachusetts.

Afternoon Session. Chairman, Leonard C. Mead, Office of Naval Research, Sands Point, L. I., N. Y.

"Problems Relating to Air Crews in Air Transport Design," by Ross A. McFarland, Research Division, Graduate School of Business Administration, Harvard University, Cambridge, Massachusetts.

"Some Considerations in Aero-Medical Research," by Lynn S. Beals, Jr., Special Devices Center, Office of Naval Research, Sands Point, L. I., N. Y.

"Human Efficiency as a Function of Light and Illumination," by Henry H. Hausner, College of Engineering, New York University, New York, N. Y.

"Theory and Methods for Analyzing Errors in Man-Machine Systems," by Alphonse Chapanis, Department of Psychology, The Johns Hopkins University, Baltimore, Maryland.

"Better Cockpit Lighting," by Clifford P. Seitz, Special Devices Center, Office of Naval Research, Sands Point, L. I., N. Y.

Tuesday, May 11

Morning Session. Chairman, Renato Contini, College of Engineering, New York University, New York, N. Y.

"The Basic Pattern of Human Locomotion," by Herbert Elftman, College of Physicians and Surgeons, Columbia University, New York, N. Y.

"An Evaluation of Experimental Procedures Used in a Fundamental Study of Human Locomotion," by Howard D. Eberhardt and Verne T. Inman, University of California, Berkeley, California.

"Recent Developments in Lower Extremity Prostheses," by John Catranis, Catranis, Inc., Syracuse, N. Y.

"Biomechanical Studies of the Upper Limbs," by Craig L. Taylor and A. Blaschke, Department of Engineering, University of California, Los Angeles, California.

"Human Engineering Problems in Service Testing of Prosthetic Devices," by Lawrence E. Abt, Research Division, College of Engineering, New York University, New York, N. Y.

"A Preliminary Experiment on the Effect of Instrument Size and Graduation on the Speed and Accuracy of Scale Reading," by William E. Kappauf and William M. Smith, Department of Psychology, Princeton University, Princeton, N. J.

SECTION OF BIOLOGY

CONFERENCE ON "NEWER SYNTHETIC ANALGESICS": MAY 14 AND 15, 1948

The Section of Biology held a Conference on "Newer Synthetic Analgesics." Doctor M. L. Tainter, Sterling-Winthrop Research Institute, Rensselaer, N. Y., was the Conference Chairman in charge of the meeting.

The program consisted of the following papers:

Friday, May 14

Morning Session. "Greetings and Historical Introduction" by the Conference Chairman.

"Chemistry of Natural and Synthetic Analgesics," by Lyndon F. Small, National Institute of Health, Bethesda, Maryland.

"Experimental Methods for Studying Analgesia," by Carl C. Pfeiffer, University of Illinois, College of Medicine, Chicago, Illinois.

"A Critique of Analgesic Testing Methods," by Lloyd C. Miller, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

Afternoon Session. Chairman, Raymond N. Bieter, University of Minnesota, Medical School, Minneapolis, Minnesota.

"Pharmacology of Metopon and Other New Analgesic Opium Derivatives," by Nathan B. Eddy, National Institute of Health, Bethesda, Maryland.

"Pharmacology of Demerol and its Analogues," by Frederick F. Yonkman, Ciba Pharmaceutical Products, Inc., Summit, N. J.

"Pharmacology of Methadon and Related Compounds," by K. K. Chen, The Lilly Research Laboratories, Indianapolis, Indiana.

Saturday, May 15

Morning Session. Chairman, Hans Molitor, Merck Institute for Therapeutic Research, Rahway, N. J.

"Animal Experimentation in Studying Addiction to the Newer Synthetic Analgesics," by M. H. Seevers, Department of Pharmacology, University of Michigan, Ann Arbor, Michigan.

"Methods and Results of Studying Experimental Human Addiction to the Newer Synthetic Analgesics," by Harris Isbell, U. S. Public Health Service, Federal Security Agency, Lexington, Kentucky.

"The Importance of Addiction to the New Synthetic Analgesic in Human Therapy," by Robert C. Batterman, New York University, College of Medicine, New York, N. Y.

"The Federal Food, Drug and Cosmetic Act and New Analgesic Drugs," by E. E. Nelson, Food and Drug Administration, Federal Security Agency, Washington, D. C.

"Regulatory Problems Under the Narcotic Law of the New Analgesics," by H. J. Anslinger, Commissioner of Narcotics, Bureau of Narcotics, Treasury Department, Washington, D. C.

Afternoon Session. Chairman, E. A. Rovenstine, New York University, College of Medicine, New York, N. Y.

"Use of the New Synthetic Analgesics in Internal Medicine," by Raymond N. Bieter, University of Minnesota, Minneapolis, Minnesota.

"Use of the New Synthetic Analgesics in Surgery," by E. A. Rovenstine.

"Use of the New Synthetic Analgesics in Obstetrics," by Bert B. Hershenson, Boston Lying-in Hospital, Boston, Massachusetts.

"Preliminary Experiences in the Use of Some of the Newer Analgesics in the Relief of Pain Due to Cancer," by R. L. Houde, L. H. Rasmussen, J. S. LaDue, and Frederick S. Phillips, The Sloan-Kettering Institute for Cancer Research, New York, N. Y.

CONFERENCE ON "ANTIBIOTICS DERIVED FROM *BACILLUS POLYMYXA*": MAY 21 AND 22, 1948

The Section of Biology held a Conference on "Antibiotics Derived from *Bacillus polymyxa.*" Doctor Perrin H. Long, The Johns Hopkins University School of Medicine, Baltimore, Maryland, was the Conference Chairman in charge of the meeting.

The program consisted of the following papers:

Friday, May 21

Afternoon Session. "Introductory Remarks" by the Conference Chairman.

"Historical Aspects," by P. G. Stansly, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut.

"Production and Isolation of Polymyxin," by J. N. Porter, G. Krupka, and

R. Broschard, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

"Effect of Various Factors on the Production of Polymyxin," by R. G. Benedict and F. H. Stodola, Fermentation Division, Northern Regional Research Laboratory, Peoria, Illinois.

"Aerosporin, Introduction," by G. Brownlee, The Wellcome Physiological Laboratories, Kent, England.

"Comparative Biological Studies of Polymyxin and Aerosporin," by H. J. White, C. Alverson, M. J. Baker, and E. R. Jackson, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut.

"Comparative Biological Studies of Aerosporin and Polymyxin," by G. Brownlee, S. R. M. Bushby, and I. E. Short, The Wellcome Physiological Research Laboratories, Kent, England.

Evening Session. "Chemical Comparison of Polymyxin and Aerosporin," by P. H. Bell, J. P. English, R. G. Shepherd, R. Winterbottom, J. F. Bone, C. E. Fellows, K. S. Howard, and M. M. Rogers, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut; and A. C. Dornbush, S. Kushner, and Y. Subba Row, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

"Comparative Chemical Studies of Aerosporin and Polymyxin," by J. R. Catch, T. S. G. Jones, and S. Wilkinson, The Wellcome Physiological Research Laboratories, Kent, England.

"Infrared Studies," by R. C. Gore, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut.

"Pharmacology of Polymyxin," by Morton F. Bryer, Emanuel S. Schoenbach, and Eleanor A. Bliss, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

Saturday, May 22

Morning Session. "Pharmacology of Aerosporin," by G. Brownlee, S. R. M. Bushby, and I. E. Short, The Wellcome Research Laboratories, Kent, England.

"Polymyxin Assay Procedures," by E. T. Reese and G. M. Eisenberg, J. T. Baker Chemical Company, Phillipsburg, N. J.

"A Simple Assay Method for Polymyxin in Blood and Urine," by P. G. Stansly, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut.

"Experimental Treatment of *Brucella suis* in Guinea Pigs with Polymyxin," by C. Larson and B. N. Carle, National Institute of Health, Bethesda, Maryland.

"The Clinical Use of Polymyxin," by Emanuel S. Schoenbach, Morton S. Bryer, and Perrin H. Long, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

"Remarks on Clinical Results with Aerosporin," by G. Brownlee, The Wellcome Physiological Research Laboratories, Kent, England.

CONFERENCE ON "THE MECHANISMS OF CELL DIVISION": MAY 28 AND 29, 1948

The Section of Biology held a Conference on "The Mechanisms of Cell Division." Doctor M. J. Kopac, New York University, New York, N. Y., was the Conference Chairman in charge of the meeting.

The program consisted of the following papers:

Friday, May 28

Morning Session. Chairman, Robert Chambers, Marine Biological Laboratory, Woods Hole, Massachusetts, and New York University, New York, N. Y.

"Cleavage in Marine Eggs," by Edwin G. Conklin, Princeton University, Princeton, N. J.

"Cell Division with Special Reference to Cells in Tissue Culture," by Warren

H. Lewis, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania.

"Cytoplasmic Changes During Cell Division with Reference to Mitochondria and the Golgi Substance," by A. J. Dalton, National Cancer Institute, Bethesda, Maryland.

"Experimental Control of Fission in *Amoeba proteus*," by H. W. Chalkley, National Cancer Institute, Bethesda, Maryland.

Afternoon Session. Chairman, E. Newton Harvey, Princeton University Princeton, N. J.

"Structural and Kinetic Aspects of Cell Division as Revealed by Micrurgical Methods," by Robert Chambers, Laboratory of Experimental Cell Research, Marine Biological Laboratory, Woods Hole, Massachusetts.

"The Action of Hydrostatic Pressure on Cell Division," by Douglas Marsland, New York University, New York, N. Y.

"Cleavage in Centrifuged Eggs and in Parthenogenetic Merogones," by Ethel Browne Harvey, Princeton University, Princeton, N. J.

"The Effects of Ultracentrifugal Force on the Cell, with Special Reference to Cell Division," by H. W. Beams, State University of Iowa, Iowa City, Iowa.

Saturday, May 29

Morning Session. Chairman, Austin M. Brues, Argonne National Laboratory, Chicago, Illinois.

"The Action of Colchicine on Cell Division in Human Cancer, Animal and Plant Tissues," by Michael Levine, Montefiore Hospital, New York, N. Y.

"The Inhibition of Cell Division by Substituted Phenols, with Special Reference to the Metabolism of Dividing Cells," by G. H. A. Clowes, Lilly Research Laboratories, Indianapolis, Indiana.

"The Action of Nitrogen Mustards and Related Substances on Cell Division," by Jonas S. Friedenwald, Wilmer Ophthalmological Institute, Johns Hopkins Hospital, Baltimore, Maryland.

"The Action of Podophyllin and its Fractions on Marine Eggs," by Ivor Cornman, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

"The Effects of Some Mitotic Inhibitors on Tumor Cells," by Ross C. MacCardle, National Cancer Institute, Bethesda, Maryland.

Afternoon Session. Chairman, G. Failla, Columbia University, New York, N. Y.

"The Effects of External and Internal Radiation on Cell Division," by Austin M. Brues, Argonne National Laboratory, Chicago, Illinois.

"The Effects of Ultraviolet Radiation on Cell Division," by Alexander Hollaender, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

"Radiation Sensitivity of Cells During Mitotic and Meiotic Cycles, with Emphasis on Possible Cytochemical Changes," by Arnold H. Sparrow, Brookhaven National Laboratory, Associated Universities, Inc., Upton, Long Island, N. Y.

"The Probable Significance of Ultrastructures in Cell Division—a Résumé," by M. J. Kopac, New York University, New York, N. Y.

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